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## Class structure in science

SHOULD universities be more responsive to the needs of industry, or is it their job to pursue learning wherever it takes them and to respond to student needs whatever they be? These questions have been asked with increasing insistence in recent months, although probably as a substitute for the more fundamental question: why does industry not appeal more to university graduates, particularly science graduates?

This is hardly simply a British problem; there has been violence in the streets in France recently over moves to direct students into courses with a higher technology content to satisfy French industrial needs. It does, however, manifest itself acutely in Britain. Class distinction and class warfare are an ubiquitous constituent of the British scene, leading to rigid boundaries and ignorant contempt. It is difficult to escape the conclusion that science has its class system too, with universities looking down on the scientific civil service, which in its turn looks down on industry. And mobility, surely the main way of breaking down a class structure, is declining all the time as jobs in universities and the civil service, with their prized security of tenure and excellent pension schemes, become increasingly scarce. Coupled with this, industries based on the physical sciences seem inexorably to be moving away from the research side of their commitment to research and development in the belief that there is not much more in basic science, as we understand it at present, which is convertible into industrial benefit. (This is a view which the Science Research Council (SRC) has also floated recently.) So the outlook is, if anything, for even more broadsides from the ramparts in the future.

It no doubt riles universities to see what they regard as a slackening regard for the pursuit of knowledge in the industrial sector. It no doubt riles industry to see universities continue to regard their best graduates as most suited to academic research. And it no doubt riles the civil service to find itself in the cross-fire and, at present, rather unloved. But the need is for a certain amount of give and take on all sides if the distinctions are not to become more rigid and more bitterly felt.

Manpower planning has a role to play in the rapprochement. Part of the argument in France has been over the payment of differential grants to favour students prepared to take technological courses. A very discrete step in this direction was made by the SRC recently in proposing that at some time in the future

it might pay certain postgraduates more than the standard grant. This has brought a predictable response from some student egalitarians, but is a sensible way of trying to raise the quality of graduates in fields where external salaries make the student who sticks to an academic course feel distinctly unequal. Now Mr Gerry Fowler, a minister at the Department of Education and Science, may have stepped back even from such tentative efforts by declaring that the matching of higher education to the manpower needs of the economy is not practicable. Indeed it is not, in a detailed way (and Mr Fowler's arguments seem aimed at detail and precision); but the case for manpower forecasting is not one which admits of too much detail, and meanwhile the half-filled science laboratories, which were built only ten years ago at government urging, are to stay half full.

These low numbers mean that the quality of university science graduates who present themselves for employment in industry is bound to decline, and industry will start to produce its own prescriptions for setting universities to rights. Just as the British workman would love to see the aristocrat reduced to productive manual labour, so industry will, with increasing fervour, call for the university to become more relevant to industrial needs. There have already been salvos from industry about postgraduate education and some of these have at least produced some sensible results. But the sort of ideas floated by Dr Marion McQuillan, of Imperial Metal Industries, in a lecture to the Research and Development Society in London last week, need to be resisted firmly. Dr McQuillan proposes that funding for university research projects should only be approved if the research has a clear economic benefit. Phrases on grant applications stating that the purpose of the work was "a better understanding of . . ." should not be acceptable. Individual universities should be designated as centres of expertise for certain industrial research purposes.

Maybe the idea was presented tongue-in-cheek. Certainly there are university departments that need a vigorous shake, but to seek to distort the function of a university so much (it would be rather like a vice-chancellor urging industry to give up profit making) serves no useful purpose beyond convincing people in universities that there is ignorance outside the walls. Up comes the drawbridge and class warfare takes another step forward.



# Dishonesty and grants

*Leigh Van Valen, Professor of Biology at the University of Chicago, is Editor of the journal Evolutionary Theory and is well known for his criticisms of "irresponsible" scientific publishing. Here he comments on an aspect of the American grant system which is also damaging to scientific research*

VARIOUS defects of the system of research grants, as it is practised in the United States, have been discussed extensively. I wish to focus on one which is rarely mentioned, although widely known, and which may well be the most serious. This is the incompatibility, in practice, between honest grant applications and conceptually original work.

The incompatibility is as follows. Any conceptually innovative scientist with some experience knows that there is a good probability that in the next year or two he will have one or more new ideas which will change the direction of his research. In practice, the National Science Foundation and the National Institutes of Health do not tolerate much deviation from the plan of research in the original proposal. The scientist must therefore either adhere to the original proposal at a loss to science, or do his best science while deviating from the conditions under which his grant was awarded.

## Two alternatives

Most scientists resolve the situation in one of two ways at the time of applying for grants:

- Original workers who do participate in the granting process use various conscious or unconscious deceptions. One kind is a proposal to do research which has just been done or will be done before the grant arrives. This is very common (M. L. Wolbarsht, *Science*, **185**, 399; 1974). Another common kind is a proposal to do research which will be done only if no new ideas come before the grant ends. For both these ploys, and others, it is empirically necessary that the nature of the ploy not be stated. This is what creates the dishonesty. When the granting agency explicitly knows of such reservations, or if the application is general enough to be honest, the application is automatically rejected. This is of course the same mentality that required J. D. Watson to falsify his NSF-administered

postdoctoral fellowship, where he did part of his Nobel Prize work that he couldn't have done if he had acted honestly.

- Research which really is predictable, at least in outline, is common and is the backbone of most scientists most of the time. There is no problem in such cases. One really can know that one will probably be able to work out the evolution of a group, or test an established hypothesis, or determine whether a known phenomenon applies in a particular case. Because the work is predictable, even though the results may not be, it is usually less than intellectually exciting. It may nevertheless have important results, and it really is necessary for the progress of science. Being easily accountable, it gets funded. Exciting work by honest people does not get funded. This may perhaps take its place among the laws of nature, at least within the boundaries of the United States.

Why should the subject of a grant be adhered to when science is better served by deviation? The administrative answer is accountability: a grant is given for a specific purpose. In applied science this is certainly appropriate. Here the purpose of the money is determined by governmental priorities, by the government's view of national (and occasionally even human) needs. In pure science, however, the aim of giving the money is the advance of scientific knowledge. It is this very advance which is hindered by the present practice. A diversity of approaches is valuable but is not permitted.

The heart of science is new ideas, and their development is discriminated against. This is not true in Europe, whatever the defects of the systems there. My European visitors, who have come from every major country, are uniformly astonished that the United States Government refuses to support creative science. The problem is as real for Szent-Györgyi (*Science*, **176**,

966; 1972), with a Nobel Prize to back him up, as it is for others. I have had several useful ideas in biology and mathematics. None were developed under a government grant, and none could have been. I have also had several grants and eventually realised that the required conformity stultified my research.

## Three categories

What to do? I suggest that there be three categories of grants:

- Most grants would be based on specific proposals, as now, but with the explicit understanding that new ideas may be followed at the scientist's discretion. Specific restrictions on such freedom may be necessary in rare cases.

- Specific proposals are inappropriate for much theoretical work; when one knows just what one will do, it is done. This is quite literally true. In such cases grants could be awarded on the basis of research done in the past three years or so, with allowance for extenuating circumstances. The second category would also be appropriate for some scientists who are beginning to work in an area different from their previous work.

- For beginning theoreticians, a modest amount of money for computer costs and the like could be provided for a year or two. These three proposals aim to minimise waste of money while endeavouring to maximise the progress of science.

Many scientists are perfectly comfortable with the present situation. Some of them are the Appollonians of Szent-Györgyi, who "develop established lines to perfection". Others are Dionysians, who know "only the direction in which [they want] to go into the unknown", but who have reconciled their dishonesty with survival. For it is often a matter of survival. Rationalisation of dishonesty is therefore easy.

We recently removed a President because he was dishonest. The norm in our science remains dishonesty, because it is made necessary for the survival of creative research. Often one may be honest or continue in science, but not both. The choice applies mainly to the more creative scientists. We can easily resolve the dilemma if we so want. Do we? □



# The Soviet energy balance

**Philip Hanson** surveys Soviet energy resources and assesses the USSR's energy policy as a factor crucially affecting the prospects for economic growth

OF ALL the countries in the world, the Soviet Union has the most impressive known endowment of non-agricultural natural resources. This is as true of fuel as it is of raw materials. In addition to being the world's second largest gold producer and a leading producer of almost all the useful mineral raw materials, the USSR has all the energy options available from domestic sources: wood, peat, oil shale, coal, oil, natural gas, hydro power and uranium.

In the past few years, however, when energy supplies have become a crucial consideration in economic policy everywhere, the Soviet energy balance has begun to seem precarious. Nobody disputes that the Soviet Union still has vast "potential", or geologically indicated, reserves of the major fuels. Beyond that, however, the Soviet energy position is open to a variety of assessments.

One problem is reserves. The extent of proved reserves, on definitions comparable to those used in the West, is not clearly established. Another problem is costs. The main areas from which increases in fuel supply can come are east of the Urals. Extraction costs in the new oil, gas and coal fields of Siberia vary greatly: for some deposits costs are quite low, for others rather high. But the social overhead costs of bringing roads, housing and amenities to these areas are generally high. Once the fields are being exploited, the cost of moving Siberian energy to the user (by oil and gas pipelines, rail shipments of coal and high-voltage electricity transmission) are generally very high. The alternative of moving the users to the energy supplies is also costly. These high costs are attributable mainly to the harsh natural conditions of most of the area—notably blizzards, permafrost and swamps.

Costs and reserves are not the only contentious issues. There is a good deal of uncertainty about the demand side. How fast will Soviet energy consumption rise in the next five or ten years? Will the Soviet Union remain a net exporter of energy in general and of oil in particular? What are the chances of the West obtaining significant energy supplies from the USSR? The wide spread and frequent revisions of recent

forecasts of Soviet energy consumption in 1980 do not inspire much confidence in such forecasts. And the implications for Soviet energy supplies to the West also depend on future Soviet policies in the supply of energy to Eastern Europe, which are not easy to predict beyond 1980.

There is another problem in any assessment of Soviet energy prospects, and this concerns technology. How badly do the Russians need Western technology in order to increase their oil, gas and coal production? Do they need it enough to ensure that some of the more grandiose East-West co-operation projects in Siberia will actually materialise?

Those are the main questions. Some can be answered with reasonable confidence; others are wide open. The accompanying tables summarise recent and planned developments in Soviet energy supply in the 1970s. The plans for 1976–1980 envisage some slowdown in the growth of the primary fuels, except for coal. With petroleum output in some of the older fields in the European USSR expected to fall, this is not too surprising. Oil and gas production in West Siberia (Tomsk and Tyumen provinces) is the main source of growth in the immediate future. West Siberia produced 148 million tonnes of oil and 38,000 million cubic metres of natural gas in 1975 and these figures are

planned to reach 300–310 million and 115,000–145,000 million respectively in 1980.

The high costs and sheer physical limits to West Siberian development in the next few years, however, seem to have led the planners to accept a slight deceleration in the growth of oil and gas production. One additional consideration may have been that proven reserves were not rising fast enough. Soviet oil reserves figures are a state secret, but several recent statements by V. Shashin, the Minister for the Oil Industry, imply that new reserves proved have recently been less than the rate of extraction. There is not much doubt that the oil is there; the USSR has 37% of the world's oil-bearing land. But exploration and proving costs are high and the gap between potential and proved reserves is large. A recent US estimate is that the USSR has a relatively modest 12.1% of the world's proved oil reserves. Reserves figures for the other fuels are not secret, though up-to-date official figures are not always available. One estimate of Soviet proved, semi-proved and probable gas reserves (Soviet categories A+B+C<sub>1</sub>) at the end of 1974 is  $23.8 \times 10^{12}$  cubic metres. This represents 92 years' production at 1974 rates—an impressive position. Nonetheless, the difficulties of exploiting these reserves are considerable, and production has fallen behind plan.

Soviet energy policy has therefore shifted recently in favour of coal and nuclear power. Major increments to coal output are planned from the Ekibastuz and Kansk-Achinsk fields whose low-cost coal, produced in large part by open-cast and strip mining, can be effectively used as a fuel for thermal

**Table 1** Soviet Energy Production

|                                                                  | 1970  | Annual average growth rate (%) | 1975  | Annual average growth rate (%) | 1980 plan (mid-point of range) |
|------------------------------------------------------------------|-------|--------------------------------|-------|--------------------------------|--------------------------------|
| Oil (10 <sup>6</sup> tonnes)                                     | 353   | 6.9                            | 491   | 5.2                            | 630                            |
| Natural gas (10 <sup>9</sup> m <sup>3</sup> )                    | 198   | 7.9                            | 289   | 7.7                            | 418                            |
| Coal (10 <sup>6</sup> tonnes)                                    | 624   | 2.4                            | 701   | 2.7                            | 800                            |
| <b>Total energy</b><br>(10 <sup>6</sup> tonnes sfe) <sup>1</sup> | 1,238 | 6.0                            | 1,656 | 5.5                            | 2,158                          |
| Electricity (10 <sup>6</sup> MWh)                                | 741   | 7.2                            | 1,038 | 5.6                            | 1,360                          |

<sup>1</sup>Standard fuel equivalent in metric tonnes of 7,000 kilocalories.

Source: Soviet plan fulfilment reports and draft directives for the 1976–1980 plan, except that total energy production figures are author's own estimates.

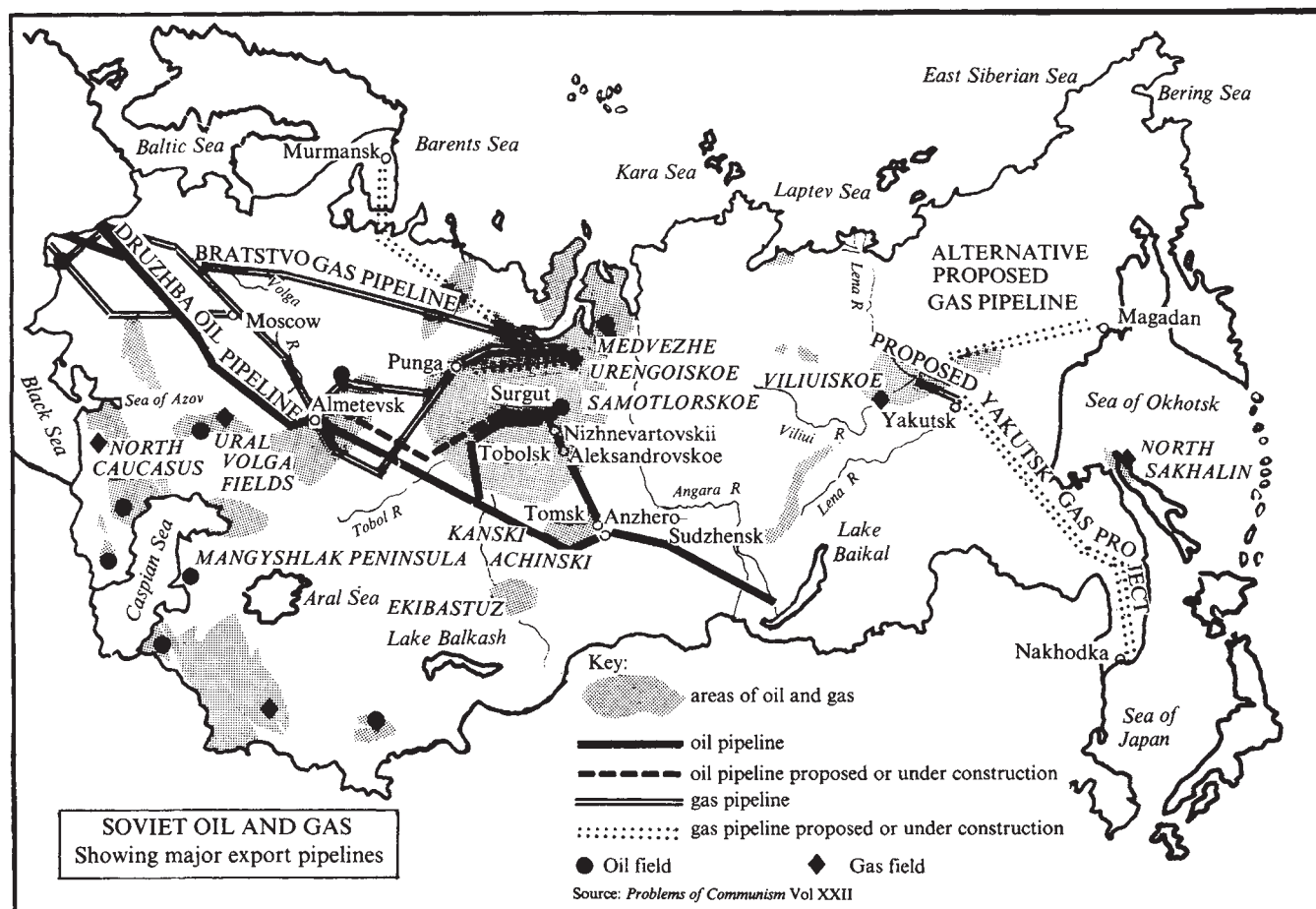
**Table 2** Contribution of Primary Sources of Energy to Total Energy Production (%)<sup>1</sup>

| Energy source         | 1970          | 1975 | 1980 plan |
|-----------------------|---------------|------|-----------|
| Oil                   | 41            | 42   | 42        |
| Natural gas           | 19            | 21   | 23        |
| Coal                  | 35            | 30   | 26        |
| Peat, oil shale, wood | 4             | 7    | 10        |
| Hydro                 | 1             |      |           |
| Nuclear               | $\frac{1}{2}$ |      |           |

<sup>1</sup>Because of rounding, columns do not all sum to 100.

Source: Author's estimates on the basis of the sources used in Table 1.





power stations in the Urals and elsewhere. At the same time the Five Year Plan requires the construction of nuclear power stations in the European USSR and the Urals with total capacity of 13–15 million kW, out of a construction programme of 67–70 million kW of new generating capacity of all types. Soviet installed nuclear power capacity has hitherto been small—3.7 million kW at the end of 1974 compared to 30.4 million in the USA and a total of about 14 million in Western Europe—so this plan would increase the role of nuclear power stations considerably.

This shift in policy has been indicated in a number of Soviet sources over the past two years. It appears to be a response both to higher world energy prices following the Yom Kippur war and to previously unexpected costs and bottlenecks in Siberian oil and gas developments. In his report to the XXV Party Congress in February, Mr Kosygin put the change in quite striking terms: future increases in oil and gas output would be allocated to “technological” needs (for example, feedstocks for the chemical industry), rather than to electricity production. The latter will depend much more heavily on hydro and nuclear power and coal than had previously been planned.

It could well be that the Soviet

planners now see energy supply as a bottleneck restricting Soviet economic growth. American recalculations of recent Soviet growth in terms comparable with the measurement of economic growth in the West show an annual growth rate of Soviet real GNP in the first half of this decade of 3.9%. The plan for the latter half of the decade (of a 4.4 to 5.0% annual growth in net material product) probably implies something like a 3½% GNP growth rate. This is admittedly only a modest deceleration, and the plan targets that are finally approved may even show no deceleration at all. But in the light of postwar Soviet plans generally, the 1980 targets look a lot more modest than they do in relation to recent performance; it is likely that they reflect newly-perceived constraints of some severity.

In the early 1970s Soviet electricity and total energy production grew appreciably faster than total national output. In part this reflected the fact that the net export of energy grew in most of the past five-year period. It probably also reflected a tendency for energy consumption to rise somewhat faster than GNP. If Soviet energy consumption continues to rise slightly faster than GNP, the exportable surplus may not grow at all between 1975 and 1980.

A recent study by Jeremy Russell

projects a Soviet energy surplus in 1980 of 200–250 million tonnes of conventional fuel equivalent; a surplus of the same order of size on that of 1975. For oil alone the surplus is likely to narrow. Russell’s conclusion is that the Comecon group as a whole will still have a surplus of oil production over consumption in 1980 but that a Comecon oil deficit, that is, net imports, is likely fairly soon thereafter.

Soviet long-term oil delivery contracts to Eastern Europe up to 1980 have already been agreed. It is likely that in 1980 only 20–30 million tonnes of Soviet oil (according to Russell), or perhaps 35 million tonnes (according to the Brussels market research organisation, *east-west*), will be available to Western markets. The USSR itself might remain a small net exporter of oil, and some imported Middle East oil might be routed to Eastern Europe through Soviet pipelines.

The position could be quite different as far as natural gas is concerned. Russell projects an annual net Comecon export of natural gas—essentially Soviet natural gas—of perhaps 30–50,000 million cubic metres a year in the early 1980s. Soviet natural gas is already being delivered by pipeline to Austria, Italy, West Germany and France, and Iranian natural gas is being piped through the USSR to West Germany, France and Austria. These

are both long term deals and could involve natural gas supplies of the order of 20,000 million cubic metres a year to Western Europe in 1980. The Siberian gas deals that have been discussed with US and Japanese companies in recent years (the Yakutsk and North Star deals) are larger and considerably more problematic. They involve tanker deliveries of liquefied natural gas out of a Pacific coast port and Murmansk, respectively, at a total rate of perhaps 50,000 million cubic metres a year over 25 years. The commercial and political obstacles to the eventual implementation of these deals are formidable. On March 31, however, a Soviet-US-Japanese agreement on joint exploration of the Yakutsk gas deposits was signed, with credits of \$25 million each being contributed by the Japanese and US consortia.

The fact that this preliminary agreement has been signed despite all the obstacles indicates the strength of both Soviet and Western interests in the development of Siberian energy supplies. The Western interest requires no explanation, but the Soviet interest is

less immediately comprehensible. Why mortgage substantial future energy outputs to the West when energy supplies already form a bottleneck to your own economic growth?

The answer seems to be that Soviet energy production can grow much faster if Western technology is used, and the gains should be enough to repay Western suppliers (in gas) and still have something left over for domestic needs. Soviet technical deficiencies are beginning to show up in a number of key areas. The items which the Russians are unable to produce domestically in sufficient quality and/or quantity in the near future include wide-diameter (1220 and 1420 mm) oil and gas pipeline, pipeline compressor equipment, machinery for deep drilling and secondary oil recovery, submersible pumps, offshore rigs and a wide range of coal mining equipment. It is likely that at least three-quarters of the 20,000 km of wide-diameter oil and gas pipeline laid in 1971-1975 was imported Western pipe. Costly pipe imports continue: in January and February about \$500 million worth of new orders

were placed in Italy, Japan and France.

The picture of pervasive East-West interdependence which these projects evoke is misleading, however. Soviet natural gas deliveries to West Germany provide less than 15% of West German natural gas consumption. The corresponding figure for the USA in the early 1980s in the event (now remote) of both the North Star and Yakutsk projects going ahead has been put at about 10%.

On the Soviet side, the best assessment seems to be that the Russians will obtain an increase in energy supplies sufficient to support a respectable economic growth rate by a mixture of policies: shifting towards fuels less efficient than oil, incurring very large domestic development costs in Siberia, making some use of Western machinery and know-how and perhaps importing significant amounts of Middle Eastern oil. Neither Soviet nor Western energy-supply problems are sufficiently awful to ensure peace on earth and goodwill between Moscow and Washington. □

## BRITAIN

# Wave energy on

*Research in Britain on alternative sources of energy took a step forward last week. Allan Piper reports*

THE first real move towards exploiting one of Britain's unconventional energy sources has come with last week's announcement that the Department of Energy (DEN) is to spend £1.01 millions on a 2-year feasibility study of wave-power devices. If the programme is successful further research could lead to the development of such devices to provide up to half of Britain's electrical energy requirements early next century.

News of the venture coincided with the publication of the latest annual report on UK offshore oil and gas resources, which gives estimates that proven reserves of North Sea oil have increased by almost a third over the past year. Although the report revises the production forecast for 1980 slightly downwards, the new estimates are seen as offering the prospect of Britain attaining self-sufficiency in energy by the end of the decade, at the same time providing a stopgap until alternatives such as the wave-power scheme also come on stream.

The decision to press ahead with work on wave power is based on a National Engineering Laboratory report identifying it as a particularly

promising energy resource. Early measurements indicate that the amount of wave energy available around British coasts varies between 40 and 70 kW per metre. The most abundant supplies are offered off north-western Scotland, but areas around the Cornish coast also show considerable potential. One advantage of wave power over other novel energy resources is that the wintertime peak of supply will coincide with maximum consumer demand.

The feasibility study is to assess the relative merits of four possible devices. Three of them, including the "Salter Duck", are British designs; the fourth is Japanese. All use different methods of converting wave energy to a usable form. Each one will be studied at a one-hundredth scale by teams of academic and industrial scientists. While looking principally at operational efficiencies and seaworthiness under varying conditions, they will also estimate approximate costs for any further research and development leading to sea-going trials.

A support programme will cover the collection and analysis of wave data, the long term effects of prolonged wave action on the structures, anchoring and mooring problems, likely environmental and navigational effects of large-scale installations, and possible modes of power generation and transmission.

The Central Electricity Generating Board has already started research into ways of integrating the supply into the national grid system.

Commenting on the decision to proceed with wave-power research, Dr Walter Marshall, Chief Scientist at the DEN, said that his department would retain control over the research teams through its Harwell-based Energy Technology Support Unit. The bulk of the £1 million will go towards the principal research programme; it will not be spread equally, but Dr Marshall gave no clues as to whether any of the devices had yet emerged as a clear favourite.

Stressing that the programme represented only an early exploratory phase of wave-power research, Dr Marshall said that further development depends on the findings of the next two years. If all goes well a 10 MW prototype should be in the sea by 1986, with a full-scale model operating by the 1990s, but Dr Marshall declined to forecast a firm date for commercial power production, as past estimates of time-scales on such major projects have generally been "hopelessly optimistic". He did say, however, that commercial installations could be operating during the next century, and DEN figures show that a 600 mile chain of devices around north-western Scotland could eventually provide about half of Britain's present electrical energy requirements even on conservative estimates. Individual devices, each up



to about 30 m across, will probably be linked together in 50-km lengths.

International interest in the project has come from the International Energy Agency, which has asked Britain to take the lead in this area of research. The only other country so far seriously involved with wave power is Japan, but Dr Marshall estimated that the total British expenditure—about £1.25 millions including independent research funding—is around four times higher than in Japan. The figure must, however, be seen in perspective: the DEN could spend almost £125 million on nuclear energy this year alone. All the same, at this early stage, Dr Marshall said, any further spending on wave power is unlikely to contract the time-scale of research.

While the wave-power decision re-

presents a significant Government step in a new energy direction, the DEN is also keeping its options open on other alternative sources of energy—solar, tidal, geothermal and wind. It is already engaged on two low-cost areas of research into a possible tidal barrage on the Severn Estuary between Wales and Somerset, and Dr Marshall has expressed a keen interest in solar power.

Even so, it seems clear that the DEN still regards the nuclear, coal, and oil and natural gas options as the mainstays of any future national energy strategy. Dr Marshall believes that the overall cost of wave-power installations could double those of nuclear generators providing comparable power, and his department remains committed to the fast breeder reactor. Additional hopes are pinned on the plan to boost

coal production over the next ten years.

As for oil, the new production and reserve estimates are aimed at giving even more room for optimism than previously foreseen. Proven reserves are now placed at 1,350 million tons, compared to last year's, 1,060 million tons, and total reserves could be as high as 3,200 million tons. The production forecast, of 95 million to 115 million tons by 1980, is down slightly on earlier estimates, because of difficulties and delays in commercial development programmes. DEN figures on energy trends, also published last week, meanwhile show that total UK energy consumption fell by 7.5% over the last year. Industrial consumption was down during the last quarter of the year by 17% on the corresponding quarter of 1974. □

A STATEMENT last week from British Nuclear Fuels Ltd (BNFL), which has otherwise captured the public eye in recent months through its nuclear fuel reprocessing activity, disclosed that the UK Ministry of Defence has placed with it a contract for the production of supplies of tritium, the radioactive isotope of hydrogen (half life 12.3 years) which is a key material in the production of H-bombs.

Construction is expected to start in June, following planning permission, of a facility at BNFL's Chapelcross Works, Dumfries, Scotland, where a plant originally built for plutonium production is now operated as a nuclear station supplying power to the national grid. The reactor there will irradiate with neutrons lithium metal which will then be treated in the new plant to separate out the tritium. The plant, which will employ fifty people, should be ready by 1980.

Until now, the Radiochemicals Centre at Amersham has bought from France the tritium Britain uses for civilian purposes, while the Ministry of Defence has obtained its military supplies from the USA. The Ministry now says that a domestic source would be more convenient and would save dollars, but it is not clear whether the civilian imports will continue or whether commercial exploitation of the domestic production for export will be attempted.

BNFL itself has meanwhile faced mixed developments on the reprocessing front. The way ahead for the deal BNFL is sharing with Cogema of France to reprocess Japanese nuclear fuel recently became clearer with the resignation of electricity industry representatives from the Japanese negotiating team which has yet to commit itself following the broaden-

ing of the deal to include Cogema.

On the home front, BNFL's Wind-scale site was recently the location for a gathering of several hundred people protesting against the use of nuclear power and, specifically, the fuel reprocessing carried out there.

● If rabies should enter Britain, the threat would be to wildlife rather than the human population. *Rabies*, a report from the Office of Health Economics, urges surveillance to ensure that pets are not smuggled into the UK or allowed ashore from small boats. The danger results partly from the changing habits of an important vector of rabies, the fox, which as a suburban scavenger now comes into closer contact with domestic animals. For those at risk, meanwhile, the rabies vaccine produced in France and currently on trial in Britain could be both safer and less painful than those previously available.

The vaccine is prepared from the Pitman Moore strain of rabies virus, inactivated with  $\beta$ -propiolactone after being grown in cultures of human diploid cells. The vaccine produces high titres of rabies-neutralising antibodies, and shows promise for immunisation both before and after exposure to the disease. It represents a considerable advance on previous vaccines, produced from viruses grown in animal nervous tissue and duck embryos.

● The fluorocarbon arguments continue, and now the Department of the Environment (DOE) has published the results of a preliminary examination carried out with the assistance of Harwell, the Department of Health, and the Meteorological Office.

Based on studies ranging from the likely effects of fluorocarbons on stratospheric ozone to the health

hazards posed by an increased influx of ultraviolet radiation, the findings suggest that the continued use of fluorocarbons at 1973 rates even for another century will ultimately destroy up to 8% of the Earth's stratospheric ozone, resulting in a 16% increase in radiation reaching the Earth's surface. This accords well with the conclusion of a similar World Meteorological Report published earlier this year.

Curbs on aerosols were not recommended, but there was a call for manufacturers and industry to step up the search for serious alternatives to fluorocarbons. The DOE report stresses that with so many uncertainties remaining the need for eventual regulations cannot be discounted, though it is hoped that ongoing research will clear up many of outstanding points within a couple of years.

A Minister in the Environment Department, Denis Howell, said (perhaps arguably) that the predicted increase in radiation levels was about the same as that incurred during a move from northern England to the south coast.

● The fluorocarbon report represents just one aspect of the work of DOE scientists, and along with environmental pollution, this department's research and development teams are also busily engaged with transport planning, building research and work on natural resources. From next August they will find themselves under a new Director General of Research when the present incumbent, Mr D. J. Lyons, steps down after five years in office to make way for Dr Martin Holdgate. Dr Holdgate, currently heading the NERC's Institute of Terrestrial Ecology, has experience leading research teams in to South America.



## BRITAIN

# Drug firm depressant

*The Labour Party last week unveiled its discussion document on state participation in Britain's pharmaceuticals industry. At least one argument adduced, concerning research, is likely to be disputed. Chris Sherwell reports*

STRONG criticism of the level and direction of private sector pharmaceutical research and development in Britain, which is subsidised by the Department of Health and Social Security (DHSS), is contained in the Labour Party's consultative policy paper "Public Control of the Pharmaceutical Industry". The views form just one plank in the platform from which it argues that, in the public interest, the recently established National Enterprise Board should "as a matter of urgency" acquire "at least one" UK-owned company with a substantial interest in pharmaceuticals and use it as a base for expansion of the public sector within the industry. It also suggests that there should be "research-based planning agreements" between domestic and foreign companies and the national health service to determine, among other things, the amount of research undertaken in Britain.

This amounts to a considerably watered-down version of the original Labour proposals for wholesale nationalisation of the industry. Indeed, the working group compiling the document now describes these proposals as "impractical". Even in their moderated form, though, the latest proposals quickly received a blanket condemnation from Mr Michael Peretz, President of the Association of the British Pharmaceutical Industry.

The research aspects of the argument appear to have a fairly pivotal role: as the 62-page document, which devotes a separate chapter to the subject, says, "the long run future of the pharmaceutical industry in Britain

depends on the quality and quantity of its research and development"; the UK-based industry's "greatest asset", it adds, is its group of research scientists. Labour's overall argument for public sector control does not necessarily stand or fall on the case it presents in respect of research and development, of course. But if preliminary reactions expressed last week by researchers themselves are anything to go by, the four identifiable strands of that case do not do much to strengthen the overall argument.

The first, and perhaps most important, strand argues that, while the existing system of profit control encourages research expenditure, it is "impossible to be sure" that all such activity is socially useful. The working group says it is "gravely concerned" that key areas of research can be neglected; there is, it says, "a social case for government funding of research into remedies for conditions which are obscure or occur mainly in developing countries"; it is "open to question whether a wholly profit orientated industry always fully serves a social purpose in long term research".

Only one of the directors of research at industrial and privately-financed research laboratories consulted last week expressed any sympathy with this view—and even he refused to allow himself to be less than 98% against the proposals on this count. Similarly, three professors could see little merit in the second strand, which anticipates the view that a mechanism already exists, through the research councils, for the government to sponsor research in areas where no commercial company would take the risks.

The working group says that even with the implementation of the customer-contractor principle, there has only been a "paper transfer of ongoing work"; there must be "changes of policy over research", not wasteful

duplication of administration. The danger, it says, is that "priorities will continue as before". Only one respondent tended to agree with this, and then only because he thought the MRC was "in a mess anyway". But no respondent disputed that the mechanism was indeed there.

The Labour group's objections go further, however. It points out that any long term research effort sponsored by a government department in an area of strong social need would have to be carefully planned; but, it says, this might replicate the very divorce of long term research from industry which is currently a weakness. It admits that closer links could not be better achieved by a public sector company which was commercially orientated. But, and this is the third strand, a long term research effort sponsored by a public sector company would be "more defensible for the government", and might also make it "easier to establish closer structural links" between the DHSS, the research councils and the industry. Research staff might also prefer to work in a publicly-owned where profit-maximisation was not the only force guiding their work, it argues.

These suggestions also fell on stony ground because they seemed so much a matter of opinion. Cooperation already existed, *Nature* was told, and more bureaucracy was not wanted. As for the final strand of the argument, concerning competition and duplication in research, there was no doubt that both were vital to progress, and in fact even the Labour group acknowledges the advantages without seriously challenging them.

Beyond the idea of closer cooperation between a publicly-owned sector and existing state-sponsored research the Labour group wants wide-ranging planning agreements and a "general system of indirect public accountability and control", the objective being "to change the balance of public and private power within the industry". □

## USA

# Explosions treaty blasted

*Colin Norman reports from Washington on the controversy blowing up over the latest US-USSR nuclear agreement*

IN JULY 1974, Richard Nixon, then almost engulfed by Watergate, completed one of his last Presidential acts of international statesmanship — the signing of a US-USSR bilateral agreement outlawing the testing of nuclear

weapons with yields greater than 150 kilotons. The treaty was greeted by arms control advocates in the USA with utter dismay. They regarded it as a meaningless gesture which would do little to dampen the arms race. Last month, that treaty was extended to cover so-called peaceful nuclear explosions and the reaction has been equally negative.

The Federation of American Scien-

tists (FAS), a liberal organisation whose sponsors include a galaxy of scientific stars, has condemned the joint pact as "worse than nothing". And last week, two former high ranking government officials argued that the treaty is little more than a sham which could seriously impede efforts to prevent the proliferation of nuclear weapons to countries which do not now possess them. Such powerful opposition could delay, or even scuttle, ratification of the treaty by the Senate.

The 1974 agreement signed by Mr Nixon and Mr Brezhnev was not due to come into effect until March 31 this year, and it covered only weapons testing—the question of peaceful nuclear explosions was left aside for further negotiations. The treaty was soon heavily attacked on the grounds that the 150 kiloton limit is too high, that the two-year delay in implementing the pact would allow both sides to get in as much large-scale testing as they wanted, and that the exclusion of so-called peaceful explosions left a loophole large enough for either military establishment to shoot massive warheads through. Most critics urged the Administration to go back to the negotiating table and come up with an entirely new treaty.

That hasn't happened. Instead, the Administration has negotiated an agreement on peaceful nuclear explosions which essentially sets an upper limit of 150 kilotons for them as well, and which leaves the rest of the 1974 pact unchanged. Though the full text of the new treaty has not yet been released, Administration officials have said that it contains a provision allowing on-site inspection by foreign nationals for peaceful explosions which consist of several blasts whose combined yield is greater than 150 kilotons. Advance warning of such events would also have to be given.

Critics charged last week that the inclusion of the provisions covering so-called peaceful explosions in an already unacceptable treaty makes a bad situation even worse. Aside from the statement from the FAS, criticism came from Dr Herbert Scoville, former chief of research and development in the Central Intelligence Agency, and Adrian Fisher, Dean of Georgetown University Law School and former Deputy Director of the Arms Control and Disarmament Agency (ACDA). Fisher was the chief US negotiator for the historic 1963 Partial Test Ban Treaty which ended atmospheric testing by all nations except France and China. Scoville and Fisher fired off their criticisms during a seminar held by the Arms Control Association.

A central complaint is that the 150 kiloton threshold is so high that it poses virtually no constraint on weapons development. Both the USSR and the USA have crammed many high-yield tests into the two-year period before the treaty was due to take effect. This has enabled the United States to develop a new warhead for the Minuteman missile, and both sides now have such a vast range of tested warheads on the shelf that there's little need to conduct further high yield tests. Moreover, weapons development in the next decade or so is likely to concentrate mostly on low-yield tactical

nuclear devices, testing of which would be allowed under the proposed treaty. As an arms control measure, the treaty is therefore viewed as ineffective.

In addition, both Scoville and Fisher suggested that the high limit could impede future negotiations in other areas of arms control. The usual US posture in such negotiations is to argue that limits should be determined by the ability of verification measures to ensure that no treaty violations take place. In this case, however, it is generally agreed that the United States is capable of detecting and identifying explosions in the USSR down to about 15 kilotons. (The actual detection limit is a matter of some dispute, but nobody is arguing that the limit is as high as 150 kilotons.) Thus, Scoville argued last week that "we have lost the principle of verification from our stance on arms control".

The USA and the USSR have twice agreed to seek a treaty banning all nuclear testing—that principle is embodied in the 1963 Partial Test Ban Treaty, and in the Nuclear Non-Proliferation Treaty—but since this treaty doesn't even come close to such a measure, Fisher argued last week that it could eventually torpedo the non-proliferation treaty (NPT). "It is silly to try to get other countries to sign the NPT while the nuclear powers are continuing to test and improve their own weapons", he said.

As for the provision limiting peaceful nuclear explosions, it was negotiated as a separate entity at the insistence of the USSR, which has plans for using nuclear blasts to divert rivers and for other excavation projects in Siberia. When the 1974 treaty was negotiated, the Soviet delegation wanted no limits on peaceful explosions, but the United States eventually insisted that such an agreement would leave a gaping loophole in the threshold test ban, since it would allow large-yield weapons to be tested under the guise of peaceful explosions. The United States, it should be noted, has virtually abandoned its own peaceful nuclear explosives programme.

At least the agreement now puts an upper limit on individual peaceful blasts, but it has nevertheless run into considerable opposition for treating peaceful explosions differently from weapons tests. The FAS statement, for example, notes that "this can only encourage new nuclear powers to justify bombs as intended for peaceful uses", just as India justified its detonation of a nuclear device. Fisher took the same tack: "we could end up with 20 nuclear nations all saying they are peaceful", he argued.

The FAS also regards the inclusion of provisions relating specifically to

peaceful devices as an unwelcome prop underneath the 150 kiloton limit. "The linkage between peaceful and military uses makes doubly unlikely any reduction of the 150 kiloton ceiling. After all, such reductions would now require the Russians to reduce their peaceful limit as well", the FAS argues.

One much publicised aspect of the agreement on peaceful blasts is that, for the first time, it contains a provision for on-site inspection. Though the details of what would trigger such inspection, and what it would entail, have not yet been made public, the provision has been termed a major advance in negotiations with the USSR. Thus, Dr Fred Ikle, Director of ACDA, has said that "we consider this as a real breakthrough, from the point of view of arms control, to have been able to negotiate with the Soviets detailed arrangements for on-site verification".

But not everybody agrees. The FAS statement calls on-site inspection "a precedent whose time has passed" since seismic detection methods coupled with normal intelligence gathering "have narrowed, virtually to equivalence, the tests which can be detected but not identified". A source in ACDA also noted that on-site inspection would be limited to non-military regions in the USSR; extension of the concept to arms controls agreements involving military installations is entirely another matter.

Scoville, Fisher and the FAS have all argued that, instead of ratifying this treaty, the Senate should insist that the Administration should return to the negotiation table and come back with a treaty banning all nuclear testing. In a telephone interview last week, Fisher suggested that "in a couple of years, we should be able to negotiate a comprehensive treaty. We haven't really tried so far—and that goes for past Administrations as well".

At present, it is unclear whether opposition to the treaty, which is only just beginning to emerge, will be sufficiently strong to persuade the Senate not to ratify the measure. A check last week with staff people in the offices of several key Senators indicated that so far the matter has not been given close attention, and that few formal statements will be made until the full text of the agreement on peaceful explosions is made available.

It should be noted, however, that ratification will require a two-thirds majority vote in the Senate; in 1973, 30 out of 100 Senators co-sponsored a resolution urging the Administration to negotiate a complete, rather than a threshold, treaty. Senator Kennedy, moreover, has also denounced the terms of the 1974 agreement, which have been left essentially unchanged.



## IN BRIEF

## Japan's NPT difficulty

The Lower House of the Japanese Diet (Parliament) ratified the Non-Proliferation Treaty last week, but a Japanese nuclear arms ban along treaty lines may still be some way off. Powerful right wing opposition in the Upper House, which must approve the Bill, could see the Bill abandoned when the present Parliamentary session ends on May 24, and there seems little chance that the ratification can remain "on the table" for the necessary 30 days for it automatically to become law, since the crucial decision to extend the session by six days also rests with the Upper House. Hawkish opposition has constantly delayed ratification since Japan signed the treaty six years ago, even though it has passed through the Lower House before.

## Cancer Institute resignation

In a development which could prove very embarrassing to the US National Cancer Institute, a top official in charge of the institute's environmental carcinogenesis programmes resigned last week, charging that his department is starved of manpower and subsumed under layers of bureaucracy. The official, Dr Umberto Saffiotti, is an outspoken individual who has persistently taken strong stands on the need to remove suspected carcinogens from the environment. His resignation is likely to fuel the growing political debate about whether or not the war on cancer in the United States is paying sufficient attention to cancer prevention.

## EEC environment research programme

A five-year research and development programme aimed at introducing better control over the environment and environmental pollution has been agreed by Ministers of the EEC Nine, and to get the £6.7 millions programme under way the Commission has asked for specific research projects from Community interests concerned with most aspects of environmental pollution, reclamation, and ecological problems raised by modern methods of land use. In most cases, successful applicants will share research costs with the community. Laboratories from two UK ministries — Environment, and Health and Social Security—are expected to submit applications.

THE Federation of American Societies for Experimental Biology includes the major American professional societies of physiologists, biochemists, pharmacologists, pathologists, nutritionists and immunologists. This April, the annual Federation meeting was in Anaheim, California, a short walk from Disneyland. I now wish I had completed the project I thought of starting about ten years ago.

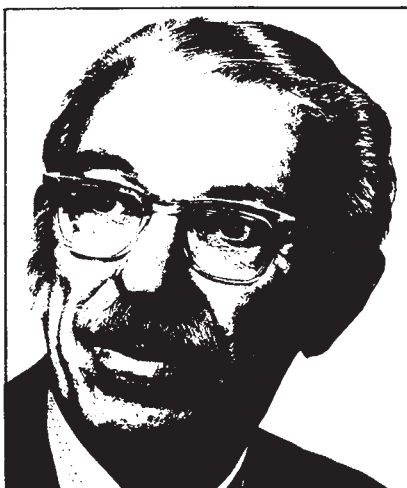
I noted then that registration at the meetings had climbed to more than 15,000, but there didn't appear to be any increase in attendance at many of the numerous scientific sessions. Some were packed, but other meeting rooms were almost empty. Where was everybody? Quite a few were at the exhibits, especially at the free Coca-Cola booth, but it seemed to me that thousands were unaccounted for. I resolved to make head counts systematically in the scientific sessions and compare them each year with the numbers registered, but I got diverted, and in any case, the Coca-Cola booth has been replaced by vending machines. I wondered about the effect of Disneyland this year as a counter-attraction to science, but I was too parsimonious to investigate the matter.

The meetings are a tribal ceremony with a rich folklore. A decent respect for my contemporaries, and a fear of reprisals, prevents me from describing the 1940 Federation meetings in New Orleans. There was a fine scientific programme, and in those lean days a front seat at the floor show in the lively Vieux Carré night clubs could be had for the price of a bottle of beer. Details may be furnished by the President of the National Academy

of Sciences, USA, if you can catch him in a reminiscent mood. He was there and it seemed to me that he didn't miss much.

After World War II, the societies grew so large that meetings were often held in the spacious accommodations at Atlantic City. Scientists

## Federation meetings



THOMAS H. JUKES

seemed more serious in those years, and diversions in Atlantic City in April are mostly unappealing. One event stands out. Convention Hall in Atlantic City is designed for major political conventions, at which a band plays on a stage in the main auditorium. The stage is skilfully constructed so that the chairman can press a button and the stage, with the band playing diminuendo, is gradually lowered into the basement by an elaborate system of electric motors

and reducing gears. The Candidate, Democrat or Republican, is then introduced to the ecstatic and partisan audience.

At the 1963 Federation meeting there was no band, of course. On stage was an eminent biochemist, Marianne Grunberg-Manago, to give an invited lecture on polynucleotide phosphorylase. Chairing the symposium was the urbane and famous Severo Ochoa, who graciously introduced Marianne. The first slide, showing how nucleoside diphosphates would form RNA-like polymers plus inorganic phosphate, came on the giant screen. The capacity audience was hushed and attentive. The chairman pressed a conveniently-placed button to dim the lights of the auditorium. Slowly and inexorably, platform, chairman, lectern and speaker descended out of sight.

Prominent scientific individualists of a type now unknown used to tread the boards at the meetings. Professor Barnett Sure had a strong dislike of the 15-minute time limitation imposed on regular papers. He would go on talking through the various warning lights and buzzers, through the discussion period, until he found the session chairman tugging at his sleeve. He would then glare at the chairman and say "First slide, please!" Many slides followed.

The American Society of Biological Chemists in 1976 decided to avoid the Federation Meetings, and are meeting separately, in June, at San Francisco, thus rejecting Mickey Mouse in favour of the adult entertainment for which San Francisco is famous. Watch for the report in *Nature*.



# correspondence

## Swine influenza

SIR,—The recent description of a human outbreak of influenza virus in an American military camp has justifiably given rise to a great deal of concern. The possibility that the virus involved might cause a severe pandemic must be seriously considered and it is perfectly understandable that vigorous measures be taken to deal with this contingency. Of such measures, the most effective is vaccination with the swine virus and the technology and resources at present available should make possible the production of sufficient quantities of inactivated vaccine for extensive use before the onset of a possible pandemic is anticipated.

Use of such a vaccine on a large scale, however, poses several problems apart from the cost of vaccine production and administration. In the first place, it may be difficult to justify the use of this vaccine for the protection of high risk subjects for whom vaccination is usually recommended, as the majority of such subjects belong to age groups in which antibody (and presumably resistance) to the swine virus is found in a high proportion of individuals due to exposure during 1918 and subsequent years of prevalence of this virus in human populations. Vaccination of younger age groups would be more justified especially considering the possibility of high mortality among young subjects as in the 1918 pandemic. It is difficult, not to say impossible, to estimate the probability of epidemic or pandemic spread of the swine virus.

This uncertainty should not dampen our efforts towards control, but what is to be our future action in the not too unlikely event of epidemics not being observed in the coming season? The risk will still be there in succeeding years and to ignore it will be unwise. If presently feasible inactivated virus vaccine is to be relied upon, the relatively short-lived protection it confers would lead to the daunting prospect of repeated early vaccination against a virus which might not appear at all. A more sensible solution would be to stockpile a strategic reserve of vaccine. Surveillance programmes in operation at present should be more than adequate to detect early signs of epidemic activity of the virus in time to enable the effective distribution and administration of vaccine. Adoption of this policy would make economic

sense as the initial investment would be compensated by savings in avoiding the unnecessary use of vaccine in non-epidemic years.

If a strategic reserve of vaccine is acceptable as a potential defence against foot-and-mouth disease of cattle, this would be preferable to the possibly superfluous use of vaccine on the massive scale anticipated in the USA or to the vaccination of already resistant subjects as proposed in this country.

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## Alternative refrigerants

SIR,—F. A. Cotton's letter (Correspondence, March 25, page 280) is an oversimplification although not, perhaps, a serious one. The fluorocarbon manufacturers estimate that the mean life of a fluorocarbon between manufacture and release to the atmosphere is about six months for an aerosol propellant and about two years for a refrigerant. The latter statement is surprising but is perhaps explained by the fact that a proportion of refrigerants is used in freeze drying and released immediately to the atmosphere, and also that in North America, at any rate, a considerable quantity is used in the air conditioning systems of cars which, perhaps sooner rather than later, are involved in head to tail collisions and release their fluorocarbons as a result.

Birks and Leck (Correspondence March 4, page 8) omitted to state that already in North America about 50% of total refrigerant used is refrigerant 22 which is unsuitable as an aerosol propellant. This contains a carbon-hydrogen bond and, for reasons not completely understood but probably because this bond is destroyed by OH radicals which it can encounter in the lower atmosphere, its lifetime in the atmosphere is rather short, probably around three years, as compared with the 80–100 year life of refrigerant 12. It thus appears that refrigerant 22 is unlikely to cause a problem as far as stratospheric ozone is concerned.

The reason commonly given by the chemical manufacturers for not using refrigerant 22 exclusively is that this would leave manufacturing capacity unused as it is not possible to manufacture R-22 in a plant designed for R-12. However, I have been informed by a particular manufacturer that he

routinely switches his plants from R-22 to R-12 according to demand. I was not previously aware that an R-22 refrigerant plant was much more expensive but, if this is so, it is difficult to understand why already half the refrigerant used is R-22.

There thus seems to be no valid reason why we should not dispense with the expensive, trivial and possibly dangerous convenience of the aerosol spray can—remembering that fluorocarbons are used almost exclusively in "personal care" products such as hair sprays and deodorants—and still retain the benefits of refrigeration.

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## Human anatomy

SIR—Kenneth Mellanby (February 19, page 523) remarked that model was a term capable of abuse, and that in Soho the word probably would not lead to useful scientific research. A week or two earlier the asymmetry of the scrotal sac of male homo sapiens had been discussed using artistic models as criteria.

Both these publications reveal the curious reluctance of scientists to research into human anatomy and relate it to function, disease and sociology. Such studies might usefully be termed gross morphological enquiries, in contrast with the vague constitutional studies which had a short flowering in the late nineteenth century and mid-twentieth century.

I have found, for instance, that asymmetry is commonplace in the human physique. The circumference ratios (right/left) of thirty right-handed women in the 18–22 years age group were measured, and averaged 1.02 (ankle), 1.06 (calf), 1.05 (thigh), 1.09 (biceps). The volume of the mammary glands also differs, although supportive garments do not allow for this fact; the result is that the right mammary gland, if supported well, implies a looser support for the left. The explanation of these asymmetries is presumably in the greater use of the muscles underlying the right hand limbs.

No evidence for this asymmetry can be found in the paintings of Titian or Rubens in the National Gallery, London or in Rodin's *The Kiss*.

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# news and views

## Revival of Milankovitch

from George J. Kukla

To support a century-old controversial theory that no one has yet been able to prove or disprove convincingly is an unrewarding business. Obviously though, something great must be in the making if the head of the UK Meteorological Office, certainly not one to suspect of holding irresponsible revolutionary views, ardently re-approves the Milankovitch theory of glaciations (see *Nature*, **260**, 396, 1976). According to the report, Mason was impressed by the coincidence in the length of orbital periodicities with those found by Hays, Imbrie, and Shackleton (unpublished) in the  $^{18}\text{O}$  records of some deep-sea cores. Geologists will be pleased but hardly surprised by the convincing answers of the computerised correlation. Fifty years ago, Eberl (*Die Eiszeitenfolge im Nordlichen Alpenvorlande*, 1930) demonstrated that the number of alpine Pleistocene terraces coincides with Milankovitch minima and 10 years ago, Broecker, (*Science*, **151**, 299; 1966) summarised available isotopic chronologies of Late Pleistocene and reported a remarkable resemblance to insolation curves. As for the energy involved, Mason sees the quantities in the "right ball park" which contradicts a view held by Shaw and Donn (*Science*, **162**, 1270; 1968). Now Weertman in this issue of *Nature* (page 17) demonstrates that Milankovitch variations are large enough to set the continental ice sheets into motion. But this is possible only if precipitation in high latitudes is

much greater than today.

Here is where the problem begins: to find the details of the mechanism through which insolation changes climate. Although today the astronomical part of the Ice Age theory is fairly reliable, Koppen and Wegener's original ideas on the direct impact of insolation on glaciers are, in the light of modern climatology, naive at best. Redistribution of energy in the Earth-atmosphere system, which operates through a number of delicate feedbacks makes the problem extremely complicated (Kellogg and Schneider, **186**, 1163; 1974).

The largest effect of orbital perturbations is in the changes of seasonal and geographical distribution of radiation over the globe. In order to influence the climate, sensitive areas and seasons must be affected by insolation to a greater degree than in the rest of the year (Kukla, *Nature*, **253**, 600; 1975). Finding these zones and intervals is critical for understanding climate dynamics. No valid explanation of past climates or successful seasonal forecast is possible without first solving this key problem. Subdivision of yearly insolation into only summer and winter segments or simple global circulation models incapable of handling subtle feedbacks, can hardly provide an answer.

Suarez and Held (of Princeton University's Geophysical Fluid Dynamic Laboratory) used an energy balanced model which simulates the seasonal

cycle and produces a large change in the extent of permanent snow and ice when past insolation structures are applied.

Berger (*EOS*, **57**, 254; 1976) has provided us with insolation values at twelve mid-month intervals in ten degree latitudinal belts for the past million years. His calculations of August irradiation on top of the atmosphere indicate a peak ( $744 \text{ Ly d}^{-1}$ ) 8,000 years ago at  $60^\circ\text{N}$  and a minimum of  $679 \text{ Ly d}^{-1}$ , 19,000 years ago. The difference of  $65 \text{ Ly d}^{-1}$  is undoubtedly large enough to affect the surface heat balance even without the volcanic dust triggers proposed by Bray (*Nature*, **260**, 414; 1976). On the other hand, the glacial-interglacial difference of  $65 \text{ Ly d}^{-1}$  is relatively small compared with the annual amplitude of irradiation to  $60^\circ\text{N}$  latitude. Insolation drops and rises by that amount in less than 10 days every October and April.

Despite millions of data bits on every element of the Earth-atmosphere system we still know lamentably little on the weather system response to this 10 day insolation shift. Can we expect to find the answers from spotty geological evidence accompanying the same irradiation change occurring over ten thousand years? Can we hope to understand glaciation and deglaciation without understanding fall and spring? With the wealth of data already at hand shouldn't we start to look seriously into the mechanism of the seasonal cycle?  $\square$

## Control of transcription of the ovalbumin gene

from Pamela Hamlyn

THE well-characterised eukaryotic mRNAs have all been isolated from cells in which they are the predominant mRNA. The mRNA for ovalbumin is no exception being the major mRNA species in part of the hen oviduct, however, it is of particular interest since its production can be induced in immature chicks by treatment with the hormone oestrogen. On administration of the hormone there is a concomitant proliferation of tubular gland cells in the

oviduct. When the treatment ceases (withdrawal) the number of these cells drops to 10–15% and ovalbumin synthesis becomes undetectable. This system is of interest to endocrinologists and also to molecular biologists, who have used it as a model system to study the control of protein synthesis (Palmiter, *Cell*, **4**, 189; 1975).

It has been known for some time that oestrogen, after diffusing into cells, combines with a protein, and that the

complex migrates to the nucleus where it remains bound to the chromatin. This is a specific reaction; it has been shown that the hormone-receptor complex will not bind to the chromatin of non-target cells. Since the oestrogen appears to bind (by way of the receptor) to the genetic material, and since one of the observed effects is the synthesis of the protein ovalbumin, the study of the mRNA was an obvious next step.

Initially ovalbumin mRNA was



detected in RNA preparations by its ability to direct the synthesis of ovalbumin in cell-free protein synthesis systems. This method has now been superseded, however, by preparing a 'probe' for ovalbumin mRNA by transcribing the messenger with reverse transcriptase to obtain a radioactively-labelled complementary DNA. The cDNA is used to measure the concentration of mRNA sequences in immature chick oviduct cells before, during and after stimulation with oestrogen (Harris, Rosen, Means and O'Malley, *Biochemistry*, **14**, 2072; 1975). Before stimulation, ovalbumin mRNA was undetectable in oviduct cells, whereas several days' administration of oestrogen resulted in tubular gland cell formation and a build-up of ovalbumin mRNA to approximately 50,000 molecules per cell. Ovalbumin mRNA synthesis stops when the hormone is withdrawn and within 12 days the level of mRNA has dropped to 0-10 molecules per cell. These results have been used to suggest that the primary regulation of ovalbumin synthesis by oestrogen occurs at the level of mRNA transcription, that is, that the oestrogen-receptor complex on binding to the chromatin activates or 'de-represses' the ovalbumin gene so that it can be transcribed. Other interpretations of these data are possible however. Cox *et al.*, for example, have pointed out the possibility that the effect of oestrogen could be to prevent the very rapid degradation of ovalbumin mRNA (Cox, Haines and Emtage, *Eur. J. Biochem.*, **49**, 225; 1974).

To investigate further whether the oestrogen-receptor complex has a direct effect on transcription, O'Malley's group have measured the number of RNA polymerase initiation sites on chromatin isolated from oviduct nuclei at various stages of oestrogen treatment (Tsai *et al.*, *Proc. natn. Acad. Sci. U.S.A.*, **72**, 4228; 1975). They have shown that during primary stimulation the number of RNA polymerase initiation sites increases, whereas withdrawal results in a drop in the number of initiation sites.

And now O'Malley and his coworkers have asked whether the RNA synthesised *in vitro* from isolated chromatin has the same tissue specificity as the RNA isolated from whole cells over the range of ovalbumin stimulation conditions. Using a cDNA probe for ovalbumin mRNA they were able to show that in their *in vitro* system only RNA transcribed from chromatin isolated from ovalbumin target cells contained ovalbumin mRNA sequences. They have also shown that RNA polymerase was necessary to produce RNA to which the cDNA would hybridise. (This is the

usual control to eliminate the possibility that ovalbumin mRNA in the cell has been isolated as a contaminant in the chromatin preparation.) Their results indicate that in general the presence of sequences transcribed *in vitro* which contain ovalbumin mRNA mimics the pattern of *in vitro* transcribed RNA with respect to hormone dependence. The authors do point out one difference however. During withdrawal there are between 0-10 molecules of ovalbumin mRNA per tubular gland cell but the chromatin isolated from the oviduct of withdrawn chicks could support the transcription of up to 5,000 molecules per cell. The authors suggest that the cells may fail to do this *in vivo* because of deficiencies in the RNA polymerase (or associated factors). Alternatively it may be (as Cox *et al.* also suggest) that low levels of mRNA are produced but are rapidly destroyed in the absence of post-transcriptional stabilisation which is in some way brought about by oestrogen.

O'Malley and his colleagues plan to use this *in vitro* system—to separate it into its components and reconstitute it—in order to study more directly how the oestrogen receptor interacts with chromatin. The continuation of this series (this latest paper is number eleven) will be awaited with interest.

## Jovian magnetotail stretches past Saturn

from John Gribbin

IN one of those surprising discoveries that, with hindsight, should not have been a surprise at all the Pioneer 10 sensors have detected the influence of Jupiter's magnetotail some 690 million km behind the planet, beyond the orbit of Saturn. This is just about the same distance from Jupiter as the Sun is on the other side—but since the magnetosphere surrounding Jupiter has a diameter of more than 14 million km it is not so surprising that the magnetotail stretches so far—about 50 magnetospheric diameters. After all, the Earth's magnetotail has been detected 500 planetary diameters 'downstream'.

Pioneer 10 passed through this extended magnetotail in mid-March, taking a full 24 h to complete its passage through the region of space influenced by Jupiter's magnetic field, according to NASA reports. Having passed the orbit of Saturn early in February, Pioneer 10 is now well on its way out of the Solar System, while its sister craft, Pioneer 11, re-targeted by its swing past Jupiter, is crossing the Solar System to encounter Saturn in three years time. The evidence for the presence of Jupiter's magnetotail beyond

Saturn's orbit comes from the Pioneer 10 solar wind detector, which registered zero during the time that the Jovian field 'shut out' the solar wind; the possibility that a genuine lull in the solar wind coincided with the spacecraft being exactly in Jupiter's magnetic shadow remains, but hardly looks plausible.

On the face of things, the observations imply that there is a roughly cylindrical volume of space influenced by Jupiter's magnetic field stretching across a sizeable fraction of the Solar System, and probably expanding slightly at greater distances from the Sun, since the solar wind density decreases as the square of the distance from the Sun. One of the most intriguing implications is that Saturn itself must pass through Jupiter's magnetotail every 20 yr, the next occasion being in April 1981 (unfortunately, just about 18 months after Pioneer 11 reaches Saturn). John Wolfe, Pioneer project scientist at NASA's Ames Research Center, comments that "this should produce some interesting magnetic phenomena".

One puzzle is that Pioneer 10 is now six degrees above the plane of Jupiter's orbit, and if the solar wind blows radially the magnetotail should be a couple of degrees, at least, below the spacecraft. But as earlier data from both Pioneer 10 and Pioneer 11 have shown, the solar wind blows gustily even at the distance of Saturn, and this turbulence could explain the tail being blown 'upwards' by six degrees temporarily.

The continuing irony of the new discoveries about Jupiter is, of course, the way in which space probes have shown that in many ways the most similar planet to the Earth is not Venus or Mars but the giant of the Solar System, a planet which contains three-quarters of all the Solar System's planetary material and which just missed, on some theories, becoming a star. □

## Nations and numbers, 1975

from Robert M. May

FOR the past few years, the Environmental Fund in Washington DC has published an annual chart giving a country-by-country summary of the best contemporary estimates of population magnitudes, growth rates, and other demographic parameters. Some of these figures are familiar to most people, and some are not.

Because of the many sources of uncertainty and delay in obtaining and reducing census data, the figures for



1975 do not simply derive from 1975 censuses. Rather they are extrapolations from the 1973 UN *Demographic Yearbook*, itself based on data up to 10 years old; the Environmental Fund's projections forward from 1973 derive from actual growth in numbers of people (from Table 4 of the 1973 UN *Demographic Yearbook*), rather than the more theoretically derived birth and death rates given in the latest UN estimates (April 1, 1975). The most significant uncertainty is in the figures for China.

In mid-1975, the total world population stood at around 4,147 million, growing at an annual rate of 2.2%. The dominant contribution was from Asia, whose 45 countries contributed 2,407 million people, with an overall growth rate of 2.5%; the growth rates of individual countries were scattered about this weighted average rate with a standard deviation of about  $\pm 0.7\%$ . Other major geographical regions are Africa with 50 countries, 420 million people, growth rate 2.8%; and Latin America with 30 countries, 328 million people, and growth rate of 2.9%. The more developed regions are the 27 European countries, comprising 474 million people with an overall average growth rate of 0.8%; North America with 242 million and growth rate 1.0%; the USSR with 254, and 0.9%; and Oceania (comprised mainly of Australia) with 21 million people growing at 2.1% per annum. In each of these major regions, the growth rates of individual countries are scattered around the weighted mean value with standard deviations in the range 0.6–0.7; thus European rates span a range from –0.4 to 2.6, Latin American from 1.0 to 3.5, African from 1.2 to 3.8.

Of the larger countries (and henceforth I shall use "larger" to mean in excess of 10 million inhabitants), the top 1975 growth rate was Pakistan, at 3.6%. Despite its tribulations, South Vietnam was a close runner-up at 3.5, followed by Iraq and Kenya with 3.4%. In the open division of this event, regardless of the country's population, the clear winner was Kuwait with 4.8% (produced in part by immigration), from Libya on 3.8 and Rhodesia on 3.7. At the other end of the scale was East Germany with a negative population growth rate of –0.4%, followed by Portugal with –0.3, Hungary with 0.1, and Sweden and the United Kingdom on 0.2.

These different growth rates, past and present, make for great sociological differences in the countries. One important index of this is the fraction of the population which is under 15 years of age. In the world as a whole, 37% of people are under 15. By region, this breaks down into 44% in Africa, 43 in Latin America, and 40 in Asia,

## Natural selection and evolution

KREBS and May's account of social insects and the evolution of altruism (*Nature*, 260, 9; 1976) is excellent, but the first paragraph raises a controversial issue. They attack statements of the type 'adaptations evolve because they are of benefit to the species (or population) as a whole'. They go on to point out, rightly, that natural selection acts on individuals, not on populations, but they then say: "Natural selection is a matter of differential survival and reproduction of individuals (or to be precise of genes), not of species". Evolution occurs over many generations, however, and genes do not exist in isolation but are part of gene complexes to which they must be adapted. Since most organisms reproduce sexually there will be many different gene complexes in the population over several generations. So for a gene to maintain its differential survival (that is, to increase its frequency or be selected) it must be adapted to many gene complexes, in other words to the entire gene pool of the population. This is what is meant by saying that an adaptation evolves because it benefits the population as a whole.

This point is important because a number of authors, particularly American ones, interpret 'adaptations benefitting populations' as implying group selection as defined by Wynne-Edwards (*Animal Dispersion in relation to Social Behaviour*, Oliver and Boyd, 1962). I have

myself recently been criticised on these grounds (Dingle, *Science*, 188, 1105; 1975), yet I never once mentioned group selection. There is an important distinction between natural selection, which relates to individuals or gene complexes, and evolution, which relates to changes in populations or in gene pools. When people write about adaptations evolving because they benefit the population or the species, this should not be thought to imply that they do not understand Darwinian natural selection, but rather that they are stressing the integration of the genes responsible for the adaptation into the gene pool.

MALCOLM EDMUNDS

JOHN KREBS REPLIES: Edmunds is correct in pointing out that good genes are ones which not only code for good characters, but also get on well with their fellow genes. However, selection does not act on large gene complexes, because they do not survive long enough as single units. Genes last a long time, so they are the units which survive in the process of natural selection. Where genes are very closely co-adapted, they evolve very strong linkage so as to become one super-gene.

Well adapted gene pools and gene complexes are obviously a consequence of natural selection for good genes, but this does not mean that gene pools and complexes are the basic unit of natural selection.

in systematic contrast with 26% in Europe, 27 in North America, 29 in the USSR, and 33 in Oceania. The top numbers are held by Iraq and Mexico, with a staggering 48% of the population below the age of 15; a dozen other countries (mainly in Latin America) follow with 47%. At the opposite end, Hungary has 20%, and East Germany and Sweden 21%. This ratio, which may crudely be thought of as a ratio of "tax consumers" to "tax producers", has a multitude of obvious implications for the social organisation of a country, some of which have been explored by Watt (*Principles of Environmental Science*, McGraw-Hill, New York, 1974) and others.

Maudlin (*Studies in Family Planning*, 6, 30–36; 1975) has catalogued the developing countries according to the official government position regarding population growth or support of family planning. It is encouraging that 34 of these 118 countries, accounting for

2,083 million people (or 74% of the total among developing countries), have an announced policy to reduce population growth rates. In the case of India, the policy reaches back to 1952; 23 others date from the 1960s, and the remaining 10 from the 1970s. The average 1975 rate of population growth in this first category of developing countries was 2.6%. A second list of 32 countries (total population 454 million, representing 16% of people in developing countries) have official support for some form of family planning activities for reasons other than reducing population growth. The average growth rate in this category of "less alarmed" countries is 2.8%. A third category, including 52 countries (although only 270 million people, or 10% of those in developing countries) does not even have support for family planning.

It is notable that so large a fraction of the developing countries has adopted policies aimed at reducing

population growth. As stressed by Korten (*Studies in Family Planning*, 6, 178-187; 1975), there are few other ideas in human history that have been transformed from taboo to official government policy in little more than a decade. Even so, the picture is not encouraging. The survey of aims and accomplishments with respect to population growth in the 14 largest developing countries (in order, China, India, Indonesia, Brazil, Nigeria, Bangladesh, Pakistan, Mexico, Philippines, Thailand, Turkey, Egypt, Iran, Korea) by Stewart (in *Population and the New Biology*, Academic Press, New York, 1974) reveals a consistent pattern of targets which, although modest enough, are not being met. India's two-thirds cut-back in the 1974 budget for its family planning programme is the sort of economy it can least afford.

Another interesting demographic statistic is the percentage of a population that is classed as urban. This statistic is made a little fuzzy by virtue of the definition of an "urban" locality differing from country to country (see *Trends and Prospects in Urban and Rural Populations, 1950-2000*, UN, New York, 1975). Nonetheless, this figure shows interesting trends, particularly

when the estimates for 1975 are put beside those for 1950.

In 1950, 29% of the world population was urban, as against 39% in 1975. Broken down into geographical groupings, these figures (expressed as percentage urban in 1950 and in 1975, respectively) are: 23 and 44 for south-west Asia; 16 and 21 for middle south Asia; 13 and 22 for southeast Asia; 17 and 31 for east Asia; 13 and 25 for Africa; 41 and 60 for Latin America; 55 and 67 for Europe; 39 and 61 for the USSR; 64 and 77 for North America; and 65 and 72 for Oceania. Concealed within these aggregated numbers are such dramatic transformations as 28% urban growing to 63% over 25 years in Martinique, 11 to 37% in Zambia, 21 to 50% in Algeria, and many other examples of a more than 200% change in the urban fraction, particularly in Africa. Very little of this differential growth in urban populations is the result of planning. The absolute lowest urban fractions in 1975 come from Nepal (5%), followed by Tanzania (7) and Uganda (8). The most urban large country in both 1975 and 1950 was Australia (with 86 and 80%, respectively), slouch-hat jackeroo image to the contrary. □

ice and DMSO can be simultaneously sublimated under vacuum from a protein solution to leave a water-soluble 'cake', which opens up a prospect for the successful freeze-drying of microorganisms and possibly tissue cultures that do not survive freezing without a cryoprotectant. Rowe and Greiff have also shown the persistence of minute open channels in the heat sealed ends of Pyrex glass ampoules through which air and water vapour may percolate leading to deterioration of the contents. These channels can be closed simply by coating the sealed end with the synthetic rubber, neoprene. □

## Drug action at the molecular level

from S. Neidle

A symposium on this topic was held on April 12-13 at the Middlesex Hospital Medical School, London, organised by the Co-ordinating Committee for Symposia on Drug Action, under the auspices of the Biological Council.

## Conservation of microbial genetic resources

from S. P. Lapage

The Third International Conference of Culture Collections (ICCC III), was held in Bombay, from March 15-19, 1976 and was attended by 290 scientists from 29 countries. It was organised by the World Federation of Culture Collections and local organising committee, under the auspices of UNEP, Unesco, ICRO and the UNEP/Unesco/ICRO panel, IUBS, The University of Bombay and other Indian organisations.

ICCC III provided a useful forum for discussion on the role and development of culture collections in developing countries. Emphasis was placed on the preservation of strains for local use and the preservation of those local strains which might otherwise be lost to science; on the relation of culture collections to the UN agencies and to the proposed development of regional microbial resource centres, with associated culture collections that would maintain the relevant strains of microorganisms, (for example suitable strains for nitrogen fixation or food fermentation in the particular region).

Also discussed were the value of national and regional federations of culture collections, the World Data

Center and its relation to other data centres and their services in providing information on the location of the microbial genetic resources held in culture collections. The related problems of standardisation of data handling and of data coding for groups of microorganisms were also considered extensively, and it was thought necessary that the agreement of the relevant international authority for each group of microorganisms concerned (such as the International Committee of Systematic Bacteriology and International Committee on the Taxonomy of Viruses) be obtained before any system of data coding was universally recommended or adopted. The deposit of microorganisms for patent procedure in culture collections and an International Treaty proposed under the sponsorship of the World Intellectual Property Organisation were also discussed, as were the problems of identification in culture collections, and the value of culture collections in many other fields—medical, veterinary, industrial and others.

The technical aspects of preservation were also dealt with. T. W. G. Rowe (Edwards High Vacuum, Crawley, UK) announced some recent work of D. Greiff (Medical College of Wisconsin, Milwaukee) who has shown that

MOLECULAR pharmacology as an area of study encompasses a wide range of disciplines, from X-ray crystallography through membrane transport kinetics, to bio-organic chemistry. This diversity of approach was well illustrated at the recent symposium on drug action. Although the contributions covered a number of different problems, it became clear however, that many of these approaches are indeed to a large extent interdependent. Thus, "hard" results from say, kinetic studies concerning a biological receptor and the drugs which bind to it are best obtainable when the receptor itself has an established molecular structure. This point was exemplified by P. J. Goodford (Wellcome Research Laboratory), who described recent attempts to design a drug to fit a known receptor. Haemoglobin, whose three-dimensional structure is well understood, was used as a model. Diphosphoglycerate, a natural inhibitor of oxygen uptake by haemoglobin, fits into a geometrically well-defined binding cleft in the protein. Instead of synthesising series of diphosphoglycerate analogues, Goodford and his colleagues simply decided to manufacture a molecule that would fit exactly into the cleft, and have appropriate groupings to confer hydrogen-bonding donor and acceptor properties. Biological testing of the inhibitor properties of their synthetic substrates have provided results which



auger well for this novel approach.

The classical approach to systematic drug design was described by C. R. Ganellin (Smith, Kline and French). The simple concepts of structure-activity relationships, such as Hansch analysis using linear free-energy relationships, have been widely used. The limitations of these methods are now well known; however, Ganellin showed that dynamic structure-activity relationships are capable of analysing quite complex situations, such as relative ionic populations in a prototropic equilibrium mixture, and hence identifying the ionisation sites of the pharmacologically active tautomer of, say histamine. The relevance of this approach (and indeed of most others) to the identification of active conformers and tautomers was questioned by W. G. Richards (University of Oxford), who reported on his recent quantum mechanical calculations on histamine. He also emphasised the importance of considering the topography of both nuclei and electrons of drug and receptor in an active environment, in order to determine the essential conformation of the drug. The active conformation of histamine, as determined from conformational probability maps, seems to be neither that determined in the solid state by X-ray crystallography, nor that present in solution, and furthermore there is no obvious positive

charge centre in the active molecule. Although these results have to be treated with caution, their implications are of some importance.

Unsurprisingly, they provoked a lively discussion, during which some experimental support was suggested for essential conformations from structural studies on enzyme-substrate complexes. The audience, which consisted mostly of experimentalists, was doubtless relieved to hear that theoretical calculations of drug conformation are by no means infallible—indeed it is a characteristic experience that increasing sophistication in *ab initio* calculation often parallels increasingly poorer quality of results.

Magnetic resonance techniques for probing drug-receptor conformations in solution are currently enjoying a considerable vogue. Thus, J. Feeney (National Institute of Medical Research) showed how he has applied them to studies of hormonal peptides, such as oxytocin and gastrotetrapeptide. Although no direct information can be obtained about their conformations when bound to receptor sites, useful data indicating preferred modes of flexibility and intramolecular hydrogen-bonding organisation are more readily accessible. From this, Feeney and his associates have developed a "zipper" theory for the mechanism of receptor binding, whereby attachment is stepwise to a series of subsites, with each step giving a reorientation of the substrate conformation. G. C. K. Roberts (National Institute for Medical Research) presented a progress report on inhibitor bindings studied by NMR to the biosynthetically important enzyme dihydrofolate reductase. Although definitive conclusions must await an X-ray analysis, it is apparent that binding of, for example, the anti-leukaemic drug methotrexate (which is a competitive inhibitor for the natural substrate) is a complex process, which is accompanied by dynamic changes in the enzyme conformation.

Drug binding to nucleic acids, especially DNA, as receptors, has for long been a favoured field of study, doubtless partly because so much is known about the detailed structures of the nucleic acids. M. J. Waring (University of Cambridge) emphasised some of the challenging and unsolved problems in this area, particularly those concerned with binding to preferred lengths of sequence. Studies of binding with the antibiotic echinomycin, a cyclic octapeptide with two quinoxaline rings attached, have shown that this compound acts as a bifunctional intercalating agent. The two planar quinoxaline rings are believed to slot in between two base pairs, that is, with a gap of 10.2 Å. The problem of, *inter*

*alia*, apparent violation of the neighbourhood exclusion principle at high binding levels has, however, hindered a detailed understanding of echinomycin binding to DNA so far.

It is not surprising that the complexity of membrane-bound receptor proteins has meant that knowledge of them at the molecular level is relatively sparse. Thus, the achievements of E. A. Barnard (State University of New York at Buffalo; now at Imperial College) and his associates in purifying the acetylcholine receptor (molecular weight 330,000) from denervated cat muscle, were the highlight of the session on these systems. It is worth remembering that the receptor, which was purified by binding to the snake venom  $\alpha$ -bungarotoxin, exists in nanomolar concentrations in the tissue used. Results mentioned by Barnard from several laboratories have indicated the sites of receptor attachment to be the postjunctional membrane. The individual receptors are on the crest of the fold facing the presynaptic sites, and there appear to be roughly 20,000 individual molecules of protein per fold, arranged somehow within about 2,000 particles, as seen in freeze-etched electron micrographs. Clearly, this area of molecular pharmacology is destined for considerable advance in the near future. □

## Solar system origin

from David W. Hughes

An advanced study institute on the Origin of the Solar System was held in the Institute of Lunar and Planetary Sciences of the University of Newcastle-upon-Tyne between March 29 and April 9, 1976 under the sponsorship of NATO.

COSMOGONY, the study of solar system origin processes is bedevilled by the fact that only one solar system is observable. The origin and evolution of stars (which are thousands of times further away and immeasurably less accessible than the planets) is much better understood simply because the sky is full of them—of different sizes and mass and at different stages in their evolution—and comparisons between them produce an abundance of clues to the physical processes responsible for their birth, life and death. The wavy paths of Barnard's star and Epsilon Eridani as they move across the celestial sphere indicate that they probably have planetary companions. Their remoteness, however, makes it completely impossible for us to measure the mass or orbits of the planets at



## A hundred years ago

THE forty-seventh anniversary of the Zoological Society was held on Saturday last, Viscount Walden, F.R.S., the President, being in the chair. Mr. P. L. Sclater, F.R.S., the Secretary, read the report, which showed that the income (28,738l.) was greater than it had been in any previous year since the foundation of the Society. The total number of visitors in 1875 had been 699,918. The new lion house had been, as far as its main portions were concerned, completed and opened to the public. The building contains fourteen dens, the larger of which measure 20 ft. by 12 ft., the smaller being 12 ft. square. The out-door cages are to be completed by the end of July next; they will measure 44 ft. by 29 ft. Mr. Sclater desired it to be known that of the larger Felidae, the Ounce (*Felis uncia*) was a desideratum. The adoption of the report was moved by Prof. Huxley, seconded by Prof. Tennant, and carried unanimously.

from *Nature*, 14, 15-16; May 5, 1876



present. An observer on one of the planetary companions of Epsilon Eridani, for example, looking towards the Sun would see Jupiter at greatest elongation  $1.6''$  away from the Sun and as an object  $10^{10}$  times less bright. Jupiter's image would thus be completely swamped by the glare of the Sun. The centre of the Sun moving around the centre of mass of the Solar System would produce an astrometric wave of peak-to-peak amplitude  $4 \times 10^{-3''}$  which is well below present-day detection limits. So a solar system such as ours orbiting a nearby star would be undetectable. The companions of Epsilon Eridani and Barnard's star must be much heavier and/or much closer to the central star than our Jupiter or Saturn.

Bearing these difficulties in mind a group of cosmogonists (a "nebulousity" of cosmogonists might be the better collective noun considering the scant common ground between their theories) met for two weeks at the University of Newcastle-upon-Tyne to discuss their latest theories and to explain them in detail to the many research workers in related fields who had also assembled there.

The best resume of the problem was given by H. Reeves (CNRS, Saclay) who expanded on his recent paper (*La Recherche*, 6, 807; 1975). Cosmogonic theories were divided into four groups dependent on whether the Solar System was formed at the same time or later than the Sun and on whether the Solar System was formed out of interstellar material which has the same chemical composition as present-day interstellar material or from material that has been altered chemically as a result of being at one time inside a star and being heated up to temperatures near  $10^6$  K. Observational facts are used to help decide between the above options.

The facts that the deuterium/hydrogen ratio is about  $2 \times 10^{-5}$  in planetary and interstellar material but less than  $3 \times 10^{-7}$  in the Sun, the lithium/silicon ratio is 200 times smaller in the Sun than in the planets, whereas the iron/silicon ratio is reasonably the same, indicate strongly that the Solar System is made of unaltered material, thus ruling out two of the options.

Ratios between the abundances of parents and decay products in radioactive series indicate that the Earth, Moon and most meteorites solidified about  $4.6 \times 10^9$  yr ago. The  $^{12}\text{C}/^{13}\text{C}$  ratio has halved over the past  $5 \times 10^9$  yr and measurements of this ratio in cometary gas and the atmosphere and surface rocks of planets indicate that the Solar System was formed quickly, and at the same time as the Sun. So the nebulousity, parts of which condensed to form the planets, was dense and short lived.

The cosmochemistry of the Solar System, which accounts for the mean composition of the planets and its variations with heliocentric distance, again requires a massive nebula ( $\sim 10\%$  of the solar mass), opaque to radiation, with temperatures varying from 1,500 K at about 0.3 a.u. from the Sun to 200 K at 40 a.u., and pressures of between  $10^{-3}$  and  $10^{-6}$  atmospheres. This temperature variation accounts for the rocky nature of the terrestrial planets and the icy and gaseous nature of the outer planets. It must also be remembered that only about 1% of the original nebula finally coalesced into planets, 99% being lost from the system.

The Reeves scenario rules out all theories which call on cloud capture long after solar system formation, interstellar collisions and binary star origins and leaves one with the basic Laplace nebula which was first propounded in 1796. All other theories have to be carefully scrutinised in the light of the above observations.

A. G. W. Cameron (Harvard College Observatory) and H. Alfvén and G. Arrhenius (University of California) were protagonists in a fascinating debate starting from positions  $5 \times 10^9$  yr apart. Cameron concentrated on star formation (that is, birth of the Solar System), invoking a pressure-induced collapse of an interstellar cloud caused by a recent encounter with a galactic spiral arm and a nearby supernova explosion. This cloud forms a cool, turbulent, dense disk out of which large protoplanets condense and subsequently disrupt leaving, as debris, the present planets. Magnetic fields and plasma effects play no part.

Alfvén and Arrhenius pledged allegiance to the 'actualistic' principle, the present state of the Solar System in conjunction with the physical processes now acting being progressively wound backwards so that older and older times are reconstructed. In the resulting theory the Solar System condenses slowly out of a low density nebula which is partially ionised and controlled by magnetohydrodynamic processes. At this stage in the debate problems of angular momentum raised their heads. These are that a one solar mass cloud of average galactic density ( $1\text{--}10$  hydrogen atoms  $\text{cm}^{-3}$ ) and differential rotation would, if it condensed to form a star, form a star which was spinning too quickly, was too hot and too highly magnetised; the planets contain 0.14% of the mass but 98% of the angular momentum of the Solar System, and conversely satellites of planets only contain 10–20% of the planet's angular momentum.

Alfvén transfers angular momentum from the Sun to the planets using Birkeland currents similar to those act-

ing in the ionosphere and magnetosphere. Cameron's Sun is originally spinning quickly and is braked over the  $5 \times 10^9$  yr by solar wind drag. D. C. Black (NASA-Ames Research Centre) makes use of the apparent rarity of star formation and the fact that stellar rotation axes are orientated randomly with respect to the galactic plane to conclude that gas clouds only collapse if the galactic corotational angular momentum is almost completely cancelled out by the turbulent angular momentum of the cloud. M. M. Woolfson (University of York) does not have this problem as in his theory the gas pulled from a protostellar cloud when the Sun passes close by has an independent angular momentum to the Sun's.

G. Herbig (Lick Observatory, California) reviewed observations of the young T Tauri type stars and indicated how their high shrinkage rates, large-scale mass ejections, variability and apparent nebula envelopes were of considerable relevance to planetary formation.

A. J. R. Prentice (Monash University, Australia) was the only contributor to introduce directly the Titius-Bode law. His "Laplacian" theory predicted rings of planetisms at the presently observed heliocentric distances but this again leaves the problem of how and why these planetisms accrete to form one, and only one, planet from each ring. The accretion and fragmentation processes taking place between these planetisms were discussed in detail by A. W. Harris (Jet Propulsion Laboratory) and J. K. Kerridge (Institute of Geophysics, University of California). W. H. McCrea (University of Sussex) suggested that Earth-Moon-Mars and Venus-Mercury were just fission products from earlier larger planets.

Diverse ideas and suggestions were put forward by many people but the list of unanswered questions remains uncomfortably long. What do meteorites have to tell of the conditions in the nebula during the early Solar System? Opinions differed greatly as to their significance. Why didn't the Asteroids form a planet? Is the planarity and orbital directness of the present Solar System just the result of  $4.6 \times 10^9$  yr of gravitational perturbation? Is there anything apart from comets beyond Pluto? Why did meteorites undergo a second heating after formation? Just how common are solar systems like our own? The remaining questions are legion, and the debate will go on far into the future; however, this study institute at Newcastle gave the problems a good airing and introduced many people to the fascinations of this intriguing subject. Two weeks of stimulating mental activity was had by all. □

# articles

## Milankovitch solar radiation variations and ice age ice sheet sizes

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*The fluctuations in the size of ice age ice sheets are calculated using glacier mechanics and the Milankovitch solar radiation variations. The calculations are greatly simplified by considering only two-dimensional ice sheets with profiles that would be appropriate if ice obeyed the flow law of a perfectly plastic solid. The solar radiation variations seem to be large enough to account for ice ages.*

THE Milankovitch theory of the ice ages<sup>1,2</sup> is based on the long term variation in the solar radiation received at presumed critical northern latitudes during certain seasons of the year. This variation is produced by changes in the magnitude of the eccentricity of the Earth's elliptical orbit around the Sun, the precession of the Earth's axis, and by changes in the value of the tilt of the Earth's axis. The eccentricity varies between the limits of 0 and 0.06, with an average period of 93,000 yr; the angle of tilt of the Earth's axis varies between 22.1° and 24.5° with an average period of 41,000 yr; and the average, precessional period is 21,000 yr (ref. 1). Ice ages are assumed to occur when the total solar radiation for the half year that contains spring and summer at certain northern latitudes is at a deep minimum value. Interglacial periods are assumed to occur when the solar radiation has a high maximum value.

Figure 1 shows the variation,  $\Delta Q$ , over the past 300,000 yr and the next 80,000 yr of the solar radiation,  $Q$ , averaged over the high radiation half of the year at 50°N (ref. 1). Today the value of  $Q$ , measured in langley  $\text{d}^{-1}$  (1 langley (ly) = 41.85 kJ  $\text{m}^{-2}$ ), at the latitudes of 60°N, 50°N, and 40°N is 787, 847, and 892 respectively for the high radiation half of the year<sup>1</sup>. Thus the variation  $\Delta Q$  that is shown in Fig. 1, which is between the limits of +50 ly  $\text{d}^{-1}$  and -35 ly  $\text{d}^{-1}$ , is the same as that produced by shifting the land mass upon which an ice sheet may be resting southwards by up to 9.5° latitude (or ~ 1,000 km) and northwards by up to 6.7° (or ~ 750 km).

No attempt has been made so far to apply the theories of the mechanics of ice age ice sheets<sup>3</sup> or of the rates of growth and reduction of ice sheets<sup>4</sup> to determine whether the Milankovitch solar radiation variations are indeed large enough to produce the very large changes in the dimensions of ice age ice sheets. The half width  $L$  of these ice sheets varies with time between the limits of about  $0 < L < 1,000$  km. Barry, Andrews, and Mahaffy<sup>5</sup> have attempted to calculate how fast a continental ice sheet nucleated in eastern Canada might form, but they made no comparison with the Milankovitch theory. Calder<sup>6</sup> calculated fluctuations of the volume of ice age ice sheets but did so with no consideration at all of glacier mechanics. He simply assumed that the rate of increase or decrease of the ice volume was proportional to the Milankovitch variation in radiation.

An attempt is made here to see if the Milankovitch solar radiation variations are sufficient to produce large variations in the size of ice age ice sheets.

### The model

To make things simple I will consider a two-dimensional ice sheet that rests on a land surface that was flat before the ice sheet formed on it. Figure 2 shows the ice sheet in profile. The lower ice surface is not flat because of isostatic depression of the land surface. The northern edge of the ice sheet, at the border of the Arctic Ocean, is situated at  $x = -L$  and the southern edge is at  $x = L$ . The total width of the ice sheet is therefore  $2L$ . The elevation of the upper ice surface above the original position of the land surface is  $h(x)$  and the depression of the lower ice surface is  $h'(x)$ . When isostatic conditions prevail and the density of rock is taken to be three times that of ice,  $h'(x) = \frac{1}{2}h(x)$ . The total thickness of the ice is equal to  $(3/2)h(x)$ .

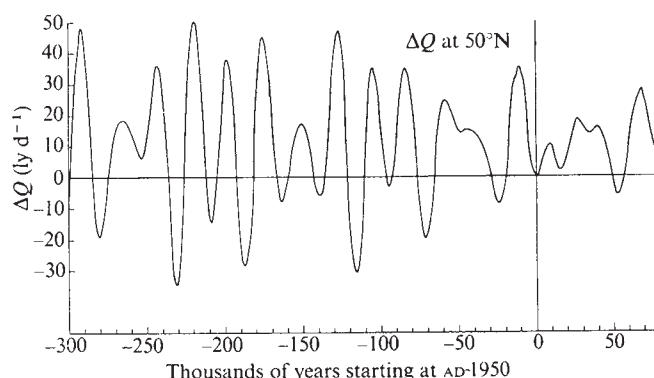
Let the ice sheet profile be approximated by that determined with the flow law of a perfectly plastic solid. The profile of an actively flowing ice sheet is given by the equation

$$h(x) = h(-x) = [\lambda(L - |x|)]^{1/2} \quad (1)$$

Here  $\lambda = 4\tau/3\rho g$ , where  $\tau$  is the yield stress which is equal to the basal shear stress ( $\sim (0.5-1) \times 10^5$  Pa) (1 Pa =  $10^{-5}$  bar),  $\rho$  is the density of ice, and  $g$  is the gravitational acceleration. The value of the constant  $\lambda$  is ~ 7-14 m.

To remain in existence the ice sheet must be nourished by snowfall. Let  $h_s(x)$  represent the snowline elevation. At elevations on the ice sheet  $h(x) > h_s(x)$  there is, over the year, a net accumulation of ice and at elevations  $< h_s(x)$  there is a net

Fig. 1 Milankovitch radiation variation<sup>1</sup>.





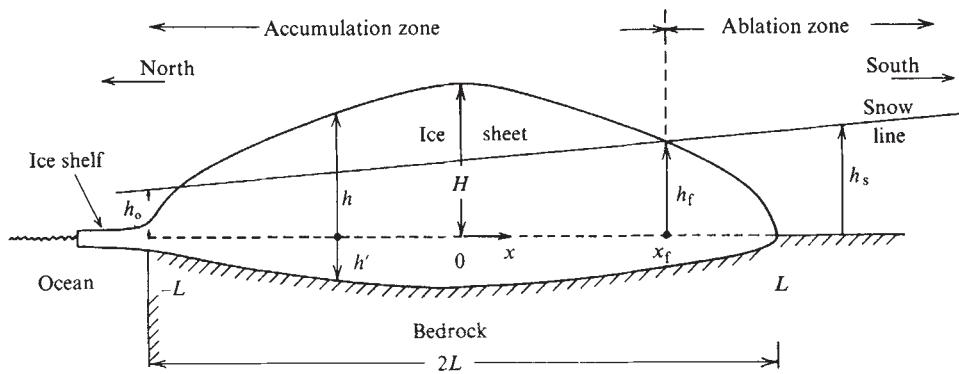


Fig. 2 Cross section of ice age ice sheet.

ablation of ice. Assume that the snowline elevation above the original land surface can be approximated by a simple linear relationship

$$h_s(x) = h_0 + s(x + L) \quad (2)$$

where  $s$  is the slope of the snowline and  $h_0$  is the snowline elevation at the northern edge of the ice sheet. The snowline to be used in any calculation is the one that would be found by placing successively larger ice sheets on the land surface and observing on these ice sheets the elevation  $h(x_f)$  at the firn line position  $x_f$  that separates the accumulation zone from the ablation zone (see Fig. 2). This snowline, which exists in the presence of an ice sheet, will be lower than the one that exists now.

### Equilibrium ice sheet

All the ice accumulated on the northern half of the ice sheet will flow out in ice shelves into the ocean as ice today flows out of the Antarctic Ice Sheet. In the southern half of the ice sheet the ice gained in the accumulation zone eventually must be lost in the ablation zone. The average accumulation rate  $\langle a \rangle$  over the whole southern half of the ice sheet is equal to

$$\langle a \rangle = [ax_f - a'(L - x_f)]/L \quad (3)$$

where  $a$  is the average accumulation rate within the accumulation zone and  $a'$  the average ablation rate in the ablation zone. Both  $a$  and  $a'$  are positive quantities.

Obviously an ice sheet is in equilibrium only if  $\langle a \rangle = 0$ . The half width  $L$  of an equilibrium ice sheet is found by setting

$\langle a \rangle = 0$  in equation (3) and then combining that equation with equations (1) and (2). The result is

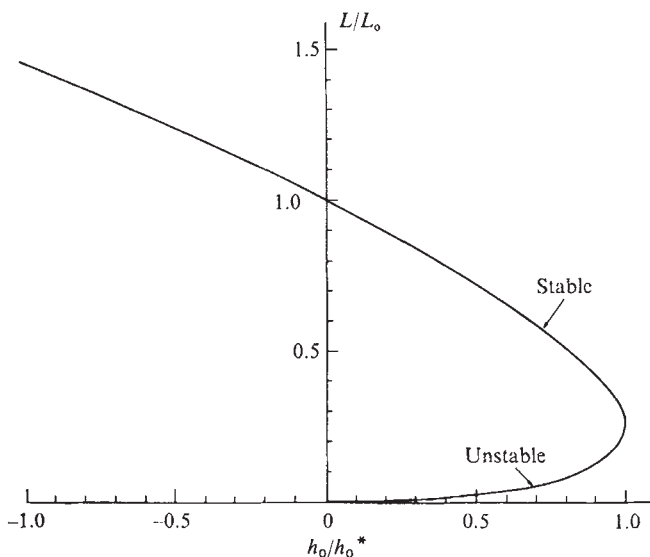
$$(L/L_0)^{1/2} = \frac{1}{2}[1 \pm (1 - h_0/h_0^*)^{1/2}] \quad (4)$$

where  $L_0 = \lambda(1 + a'/a)/s^2(1 + 2a'/a^2)$  and  $h_0^* = \lambda/4s(1 + 2a'/a)$ . Figure 3 shows a plot of  $L/L_0$  against  $h_0/h_0^*$ . Obviously no equilibrium ice sheet can exist if  $h_0 > h_0^*$ . The plus sign in equation (4) gives the half width  $L$  of a stable equilibrium ice sheet and the minus sign the half width  $L$  of an unstable equilibrium ice sheet. If  $h_0 < 0$ , only the solution of the stable equilibrium ice sheet is physically meaningful. A stable equilibrium ice sheet over time approaches its equilibrium size if its dimensions are perturbed. An unstable equilibrium ice sheet either shrinks until it disappears or grows until it reaches the stable equilibrium size if its dimensions are perturbed. The change in size of a non-equilibrium ice sheet is a consequence of the fact that  $\langle a \rangle \neq 0$ .

It should be noted that the half width  $L$  of the equilibrium ice sheet is independent of the actual values of  $a$  and  $a'$ . It only depends on the ratio  $a'/a$ . This result is not valid if a power-law creep equation is used to determine the ice sheet profile. The resulting dependence of  $L$  on  $a$  and  $a'$  is, however, relatively weak<sup>3</sup>.

The magnitude of terms  $L_0$  and  $h_0^*$  should be noted. For the not unreasonable values  $a'/a = 2.75$ ,  $s = 10^{-3}$ , and  $\lambda = 14$  m one finds  $L_0 = 1,245$  km and  $h_0^* = 0.54$  km. Now suppose  $h_0$  were to fluctuate in value between limits  $> 0.54$  km and  $< 0$  km because of Milankovitch variations in radiation. The value of the stable equilibrium half width  $L$  of the ice sheet would alternate between 0 km and a limit  $> 1,245$  km.

Fig. 3 Normalised plot of equilibrium ice sheet size against normalised snowline elevation at northern edge.



### Time variation of ice sheet size

The variation in time,  $t$ , of the volume,  $V$ , of the southern half of the ice sheet is simply

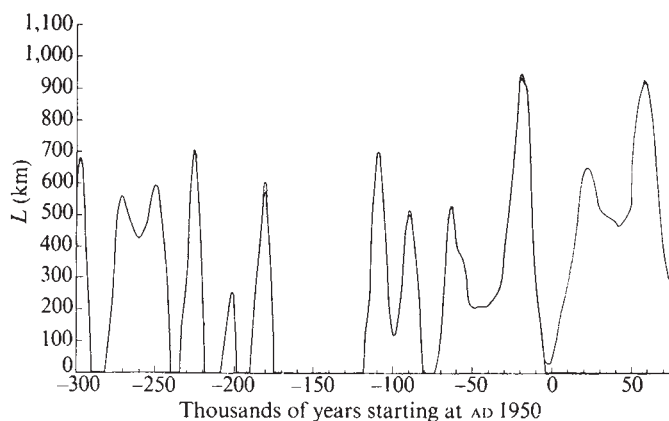
$$dV/dt = ax_f - a'(L - x_f) \quad (5)$$

For an active ice sheet  $V = (\lambda L^3)^{1/2}$  and the rate of change  $dL/dt$  is given by

$$dL/dt = (2a/3\lambda^{1/2}L^{1/2})\{L - [(1 + a'/a)\lambda/4s^2] \times [-1 + (1 + 4s[h_0 + 2sL]/\lambda)^{1/2}]\} \quad (6)$$

This equation is found by combining the preceding equations. Since for an active ice sheet  $h(x) = h(-x)$  the northern half of the ice sheet changes its size at the same rate as does the southern half.

Equation (6) applies only if the ice sheet is actively flowing, that is, the basal shear stress is equal to the yield stress. Now it could happen that the snowline is raised so that the whole ice sheet is in an ablation zone. In this situation the removal of ice reduces the basal shear stress to below the yield stress and ice no longer flows. The ice sheet is stagnant. The rate of wastage of the ice sheet can be quite rapid in this situation<sup>4</sup>. The rate of



**Fig. 4** Half width,  $L$ , of ice sheet as a function of time calculated with  $\lambda = 14$  m,  $\Delta Q_0 = 10 \text{ ly d}^{-1}$ ,  $s = 0.001$ ,  $\alpha = 0.021 \text{ km ly}^{-1} \text{ d}$ ,  $a = 1.2 \text{ m yr}^{-1}$ ,  $a'/a = 2.75$  (thick line) and  $2.745$  (thin line).

change  $dL/dt$  whenever  $h_s(0) > h(0)$  will be approximated by the equation

$$dL/dt = -2a'(L/\lambda)^{1/2} \quad (7)$$

This rate of shrinkage is three times faster than that found by assuming that the ice sheet is active.

A growing ice sheet can also be stagnant. Suppose that the snowline is suddenly lowered and  $h_0$  has a negative value. If  $-h_0/s > 2L$ , snow will accumulate on the ground ahead of the ice sheet, or an ice sheet will nucleate if  $L = 0$ . In this situation it will be assumed that either  $a$  is large enough or the term  $-h_0/s$  is sufficiently small that no serious error is introduced by setting  $L = -h_0/2s$  and  $h(x)$  the value given by equation (1).

## Connection with the Milankovitch radiation variation

We saw earlier that the Milankovitch variation in the solar radiation  $\Delta Q$  is similar to that produced by shifting the land surface north and south by distances  $\sim 1,000$  km. If the snowline were fixed in space but the land surface were shifted north and south by such distances the effect would be the same as keeping the land surface fixed and moving the snowline

elevation up and down. In other words the value of  $h_0$  would be given by the equation

$$h_0 = \alpha(\Delta Q + \Delta Q_0) \quad (8)$$

where  $\Delta Q_0$  is a constant and

$$\alpha = s/(dQ/dx) \simeq (21 \text{ km ly}^{-1} \text{ d}) \times s.$$

We assume that in this naive way the solar radiation variations can be related to changes in the elevation of the snowline.

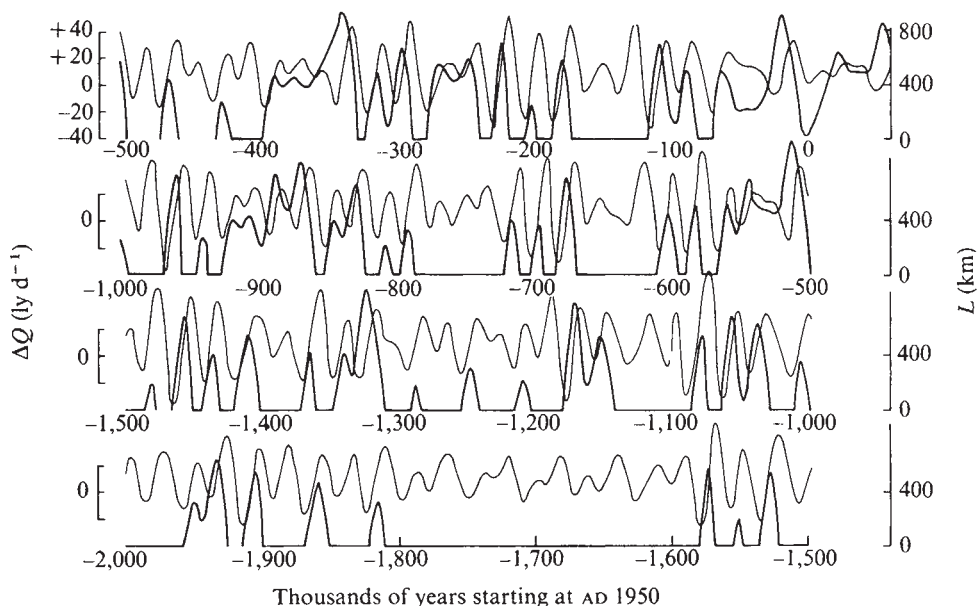
The Milankovitch radiation variations undoubtedly cause changes in the value of  $a$ ,  $a'$ , the ratio  $a'/a$  and possibly in the slope  $s$ . Only the change in size of the ice sheet produced by changes of the snowline elevation given by equation (8) will, however, be considered because there seems to be no good way of estimating how the other quantities are altered by the variations of the radiation.

I have calculated the variation of  $L$  using equations (6), (7) and (8) and the Vernekar tables<sup>1</sup>. The Vernekar tables list the value of  $\Delta Q$  at 1,000-yr intervals over the past 2 Myr and for the next 120,000 yr. The computer calculations were made incrementally by calculating  $\delta L/\delta t$  where  $\delta t$  was taken as 1,000 yr. The values of  $\Delta Q$  chosen were for  $50^\circ \text{N}$ . The value of  $L$  2 Myr ago was set equal to zero. (For Fig. 6 at 1 Myr ago.)

Figure 4 shows two plots of  $L$  over the past 300,000 yr and for the next 80,000 yr for  $s = 10^{-3}$ ,  $\lambda = 14$  m, and  $a = 1.2 \text{ m yr}^{-1}$ . Two values were chosen for  $a'$ :  $2.75a$  and  $2.745a$ . The value of  $\Delta Q_0$  was taken to be  $10 \text{ ly d}^{-1}$ , which gives  $h_0 = 0.2 \text{ km}$  at the present time. The value of  $h_0$  over the past 300,000 yr varies between the limits of  $1.3 \text{ km}$  and  $-0.5 \text{ km}$ . Figure 5 shows the same plot, for  $a' = 2.745a$ , carried back to 2 Myr and superimposed on the Milankovitch curve.

I believe that Figs 4 and 5 demonstrate that the Milankovitch radiation variations are potent enough to be the prime cause of ice ages, although these plots by no means prove that the Milankovitch ice age theory is correct. The magnitude of the highest peaks,  $\sim 1,000 \text{ km}$ , is of the right order of magnitude. The position and relative magnitudes of the peaks and troughs over the past 130,000 yr correlate well with sea-level fluctuations over the same period (see Fig. 7 of ref. 7). The main criticism that can be directed at Figs 4 and 5 is the use of a rather large, but not impossibly large, accumulation rate as well as ablation rate. At the present time the precipitation in north-eastern Canada is about half this level.

If a much smaller ratio  $a'/a$  is used the value of  $h_0^*$  becomes so large that once an ice sheet is formed it never disappears.



**Fig. 5** Same plot as Fig. 4 for the case  $a'/a = 2.745$  but for a longer time period. Also shown is the radiation variation.



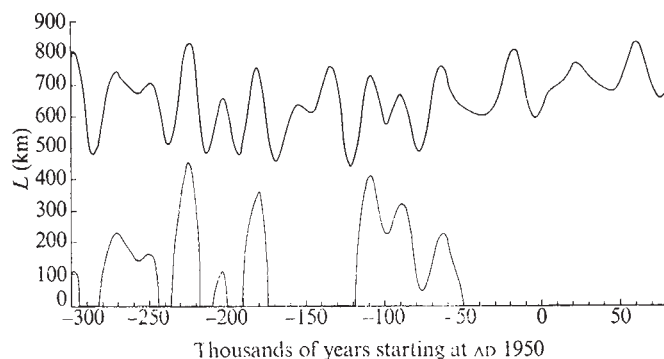


Fig. 6 Same plot as Fig. 4 but with  $\lambda = 14$  m,  $\Delta Q_0 = 10$  ly d $^{-1}$ ,  $\alpha = 0.02625$ ,  $a = 0.3$  m yr $^{-1}$ , and  $a'/a = 2.2$  (thick line) and 2.4 (thin line).

If a much larger ratio is used the ice sheet never grows to a large size. If smaller values of  $a$  as well as  $a'$  are chosen, the peak size of the ice sheet is reduced. Figure 6 shows an example of reduced peak size. This figure also illustrates a case of non-disappearance of an ice sheet. The use of  $a = 0.6$  m yr $^{-1}$  can produce peaks  $\sim L = 700$  km.

The smaller value of  $a$  produces smaller peaks in  $L$ , not because the equilibrium size of the ice sheet is small, which it may not be, but because the rate of build up of the ice sheet is smaller. The ice sheet does not approach a large equilibrium size before the value of  $\Delta Q$  changes and causes the ice sheet to waste away.

Figure 4 also demonstrates the hazard of using a Milankovitch curve to predict future ice ages as well as to determine when past ice ages must have occurred. According to Fig. 4 and data not plotted, depending on whether  $a'/a = 2.750$  or  $a'/a = 2.745$ , there will be no ice age for the next 120,000 yr or one actually

is starting to build up right now. (Note that the value of  $L$  for the thin line curve at  $t = 0$  is  $\sim L$  of existing ice caps in the Arctic.) By assuming different values of the constant  $\Delta Q_0$  new peaks can be made to appear in the plots or old peaks can be made to disappear.

## Conclusion

I believe that it has been demonstrated that the solar radiation variations that are the basis of the Milankovitch theory are large enough to have produced ice ages. To make the large ice age ice sheets that are known to have occurred, however, it is necessary that high accumulation and ablation rates existed at least on the southern half of the ice sheets—rates that are substantially higher than occur today in Greenland and Antarctica. These calculations demonstrate the rather unusual position the northern land masses must have for it to be possible for ice ages to come and go over the past 1–2 Myr. If the northern land masses were all shifted southwards by  $\sim 500$  km it would be equivalent to increasing  $\Delta Q$  by  $\sim 25$  ly d $^{-1}$ . Suppose all other factors remained constant (precipitation is not changed, for example, in spite of the altered water circulation into and out of the Arctic Ocean). After such an increase in  $\Delta Q$  it would be very difficult to give predictions for ice ages with large ice sheets using our model while at the same time requiring the model to predict alternating ice ages and interglacials with the land masses in their present position. Similarly if the land masses were shifted northwards by  $\sim 500$  km it would be difficult to make the model predict anything other than a perpetual ice age.

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# Thermal radiation produced by the expansion of the Universe

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*I show here that the expansion of the Universe can create particles with a black-body distribution. I give a simple model in which the creation of particles in an initially empty spacetime leads at very early times to a Friedmann expansion dominated by hot relativistic particles (probably including gravitons) having a black-body spectrum, even before classical thermalising processes have had a chance to act. If the particle creation occurs near the Planck time, the entropy produced is consistent, in order of magnitude, with that required by the Einstein equations. The conditions in which one achieves consistency with the observed 3K black-body radiation are also discussed.*

THE model we discuss in detail here assumes a Robertson-Walker (isotropic) space time which expands from a possibly singular state characterised by the universal constants  $G$ ,  $\hbar$ , and  $c$ . The metric is treated classically, whereas the matter and radiation are quantised. The equations of the quantised particle fields are the simplest (minimally coupled) generalisations of the special relativistic free-field equations. We assume that other

interactions have a negligible effect on the particle creation process near the singularity, compared with gravity. No particles are present initially. Near the singularity, the particle field equations for spins other than 0 and 2 are conformally invariant (the masses can be neglected because of the high energies involved), so that they lead to no particle creation in the isotropic model<sup>1–3</sup>. Mesons obeying the minimally coupled spin-0 field equation will, however, be created in abundance near the singularity. We show that these mesons have the remarkable property that they are created with a black-body spectrum, and hence will be in thermal equilibrium *ab initio*, even in regions of space time which are not causally connected<sup>4</sup>. The same can be said of gravitons obeying the linearised Einstein equations given by Grishchuk<sup>5</sup>, since each component  $h_i^k$  obeys our equation (2) when the  $\tau$ -time defined below is used. Entropy is generated because the particles are created in pairs, and correlations between members of a pair are unobservable on account of subsequent interactions and decays. Although these results do not make use of the Einstein equation which implies that the density at  $t_p$  is of the order of the Planck density, they are consistent with it<sup>5,6</sup>, and further provide a mechanism for producing the necessary entropy. It should be noted that in

the model considered here, one starts with very simple initial conditions (empty Minkowski space) and obtains a black-body spectrum at a very early time, independent of classical thermalising mechanisms. (Our results are not necessarily inconsistent with a 'bouncing' model, as in refs 6 or 7, if appropriate boundary conditions are imposed at the minimum of  $a(t)$ .)

Any mechanism consistent with the Einstein equations which yields uniform black-body radiation before  $t_e$ , the time at which the matter and radiation densities become equal, will lead to an expression for the present black-body temperature which depends on  $t_e$  raised to the one-sixth power. We interpret this expression as giving the present temperature to be approximately equal to the redshifted temperature of the initially created particles.

To obtain the present matter density, and thus  $t_e$ , from this model, one could use a matter-antimatter separation mechanism (possibilities are reviewed in ref. 19), or an interaction which did not conserve baryon number. One could also introduce the excess baryon number in the initial state, without significantly affecting the temperature. We make no attempt here to predict the entropy per baryon.

In anisotropic expansions with similar boundary conditions on the minimally coupled scalar field, we find that the particles are created with a direction dependent distribution resembling Doppler shifted radiation in an anisotropically expanding thermal cavity with perfectly smooth reflecting walls and no dust. As in the latter case, one would expect interactions to bring out a true black-body spectrum, while, as suggested by Zeldovich, the expansion itself was isotropised through the gravitational interaction of the created matter. Our results should be similar to those of ref. 8, the main modification being in the initial particle distribution. A special case of such a distribution was found by Berger<sup>9</sup> in the degenerate Kasner universe. We, however, view our present boundary conditions, incorporating  $\hbar$ ,  $c$  and  $G$ , as corresponding to a physical process occurring only when the curvature is sufficiently large, and not near a pseudo-singularity, as in the degenerate Kasner.

From a fundamental viewpoint, the thermal production of particles may point towards a generalisation of the association of entropy and temperature with black-hole space times<sup>10,11</sup> to cosmological space times, or to singularities themselves. In that connection, the cosmological thermal emission may have a role analogous to the thermal emission by black holes discovered by Hawking<sup>12</sup>.

The spectrum and probability distribution of the particles created by the isotropically expanding universe have been given before<sup>1,2</sup>, but the detailed form of the coefficients  $|\beta/\alpha|$  involved, and the connection with black-body radiation, is given here for the first time. More details, together with a treatment of the anisotropically expanding model, will be published elsewhere.

## Isotropic model

For simplicity, we consider the spatially flat Robertson-Walker metric

$$ds^2 = dt^2 - a^2(t) \sum \delta_{ij} dx^i dx^j$$

The presence of spatial curvature will not significantly alter the result. As noted earlier, there is significant particle creation only by the minimally coupled spin-0 (and spin-2) field equation  $\nabla_\mu \nabla^\mu \phi = 0$ , where  $\nabla_\mu$  denotes the covariant derivative. (We use units with  $\hbar=c=1$ .) The mass can be neglected because the energy of the created particles which contribute to the present black-body spectrum is very large compared with elementary particle rest masses. Canonically quantising the field in the usual way, we write

$$\phi(\mathbf{x}, t) = (2\pi)^{-3/2} \int d^3k [A_{\mathbf{k}} e^{i\mathbf{k}\cdot\mathbf{x}} \psi_{\mathbf{k}}(t) + \text{H.C.}] \quad (1)$$

where  $k = |\mathbf{k}|$ , and the  $A_{\mathbf{k}}$ ,  $A_{\mathbf{k}}^\dagger$  obey the usual commutation

relationships. For simplicity, we are considering the neutral field. In ref. 1 we showed that charged particles are also created (in particle-antiparticle pairs) with the same probability distribution. The functions  $\psi_{\mathbf{k}}(t)$  satisfy

$$d^2\psi_{\mathbf{k}}/dt^2 + a^4 k^2 \psi_{\mathbf{k}} = 0 \quad (2)$$

with

$$\tau = \int a^{-3}(t') dt'$$

The Wronskian condition is  $\psi_{\mathbf{k}}(d\psi_{\mathbf{k}}^*/d\tau) - \psi_{\mathbf{k}}^*(d\psi_{\mathbf{k}}/d\tau) = i$ .

## Boundary conditions

We assume that for  $t$  large with respect to  $t_p$ , the expansion parameter  $a(t)$  is given by the general relativistic solution for a universe dominated by relativistic particles and radiation, namely  $a(t) = Ct^{1/4}$ , where  $C$  is a constant which does not enter our final results. As  $t$  approaches  $t_p$ , we let  $a(t)$  join smoothly to a constant value  $a_1$  given, approximately, by  $a_1 \simeq Ct_p^{1/4}$ . In terms of  $\tau$  time, the approach of  $a$  towards  $a_1$  will be greatly stretched out and very gradual, since as  $t \rightarrow t_p$  (and  $a$  becomes small)  $\tau$  approaches very large negative values. The results will be independent of the detailed behaviour of  $a(t)$  near the 'singularity', but will depend on its being characterised by  $G$ ,  $\hbar$ , and  $c$ . We regard the smooth joining of  $a(t)$  to a constant  $a_1$  as the simplest means of constructing unambiguous initial conditions incorporating the Planck dimensions, and not necessarily as a true representation of the universe 'before' the expansion began (just as 'turning on' an interaction between particles does not imply it was ever absent in nature).

As an initial condition we suppose that for large negative  $\tau$

$$\psi_{\mathbf{k}} \Big|_{\tau \rightarrow -\infty} = (2ka_1^2)^{-1/2} \exp(-ika_1^2\tau)$$

so that the  $A_{\mathbf{k}}$  are the usual special relativistic annihilation operators corresponding to physical particles in the initial Minkowski space ( $a=a_1$ ). (The quantity  $k/a$  is the magnitude of the momentum.) We assume that no particles are present initially,  $A_{\mathbf{k}}|0\rangle=0$  for all  $\mathbf{k}$ , where  $|0\rangle$  is the state of the system (Heisenberg picture). After  $a(t)$  has settled down to the form  $Ct^{1/4}$  there will be essentially no particle production resulting from the expansion, because, as we showed in ref. 2, or as can be directly verified from equation (2), the WKB expressions for  $\psi_{\mathbf{k}}$  are exact solutions for that particular form of  $a(t)$ .

## Fundamental approximation and solution

The key characteristics of  $a(t)$  are that, viewed as a function of  $\tau$ , the quantity  $a^4$  in equation (2) gradually approaches a constant value  $a_1^4$  for large negative  $\tau$  and grows smoothly to a value large with respect to  $a_1^4$ . It is furthermore mathematically convenient (but does not affect the result, since the particle creation is negligible when  $a$  is large) to let  $a^4$  approach a constant value  $a_2^4$  for large positive  $\tau$ , so that one has a final Minkowski space in which to unambiguously determine the results. Then, following a method which has been used in treating the reflection of radio waves and quantum mechanical reflection from a potential barrier in the regime where the WKB approximation would yield no reflection<sup>13,14</sup>, we approximate  $a^4$  in equation (2) by

$$a^4 \simeq a_1^4 + e\zeta [(a_2^4 - a_1^4)(e\zeta + 1) + b] (e\zeta + 1)^{-2} \quad (3)$$

where  $\zeta = \tau s^{-1}$ ,  $s$  is a constant giving the range in  $\tau$  time over which the transition from  $a_1$  to  $a_2$  takes place, and  $b$  is a positive constant. Our final result will be independent of  $b$  and  $a_2$ . One easily sees that for the present problem  $s \simeq a_1^{-3}t_p$  (for example, in an expansion with  $a \propto t^{1/4}$  starting at  $t_p$ , the  $\tau$  interval for  $a^4$

to grow from  $a_1^4$  to  $16a_1^4$  is  $\Delta\tau = a_1^{-3}t_p$ , whereas the  $\tau$  interval for  $a$  to grow to infinity is  $2a_1^{-3}t_p$ .

For large  $\tau$ , we have

$$\Psi_k \Big|_{\tau \rightarrow \infty} = (2ka_2^2)^{-1/2} [\alpha_k \exp(-ika_2^2\tau) + \beta_k \exp(ika_2^2\tau)]$$

where  $\alpha_k$  and  $\beta_k$  are complex constants. The Wronskian condition requires that  $|\alpha_k|^2 - |\beta_k|^2 = 1$ . The annihilation operators  $a_k$  for particles at late times are given by  $a_k = \alpha_k A_k + \beta_k^* A_{-k}^\dagger$ . The exact solution of equation (2) with  $a^4$  given by equation (3) is discussed in refs 13 and 14, where it is shown that

$$\left| \frac{\beta_k}{\alpha_k} \right|^2 = \frac{\sin^2 \pi d + \sinh^2 [\pi s k (a_1^2 - a_2^2)]}{\sin^2 \pi d + \sinh^2 [\pi s k (a_1^2 + a_2^2)]} \quad (4)$$

where  $d$  is a real number involving the constant  $b$ . For any  $k$  significant for the highly red-shifted radiation observed in the present universe, the quantity  $ska_2^2$  is very large compared to unity (for example, if  $a_2 \approx Ct_p^{-1/2}$  with  $t_p \approx 10^{-28}$  s, then  $ska_2^2 \gg 1$  for all  $k$  contributing to present frequencies  $> 1$  Hz). Therefore, to an excellent approximation

$$|\beta_k/\alpha_k|^2 = \exp(-4\pi s k a_1^2) \quad (5)$$

This result is independent of  $b$  and  $a_2$  in equation (3), and depends mainly on the gradual and smooth joining to  $a_1$  which takes place at very early times. These considerations, together with general results<sup>15,16</sup> about the behaviour of  $|\beta_k/\alpha_k|$ , suggest that an expression of the form of equation (5) holds whenever the approach of  $a(\tau)$  to  $a_1$  is sufficiently smooth. (We are not yet sure whether the exponential approach of  $a^4(\tau)$  to  $a_1^4$  as  $\tau \rightarrow -\infty$  is essential; it may only be necessary that  $a^4$  and all its derivatives be continuous and that  $a_2 \gg a_1$ .) The result in equation (5) is certainly independent of the (reasonable) behaviour of  $a$  when it becomes large with respect to  $a_1$ . It should hold for the actual expansion (equation (3) was introduced for mathematical convenience) by the time  $|\beta_k/\alpha_k|$  ceases to change significantly (certainly no later than  $10^{-20}$  s, and probably much closer to  $t_p$ ).

## Energy distribution of created particles

We have shown<sup>1,2</sup> that production takes place in particle pairs, and have given the probability distribution of the created particles. From equation (51) of ref. 2, we conclude that the probability of observing  $n$  pairs, where one particle is in mode  $k$  and the other (antiparticle) in mode  $-k$ , is

$$P_n(k) = |\beta_k/\alpha_k|^{2n} |\alpha_k|^{-2}$$

Equation (52) of ref. 2, on the other hand, gives (in the discrete representation) the average number of particles in mode  $k$  in a coexpanding volume element as  $\langle N_k \rangle = |\beta_k|^2$ . The correlations between particle and antiparticle of a pair are effectively destroyed by decay processes and other interactions. In this way entropy is generated by the particle creation process, and the particles (or antiparticles) alone are described by a density matrix or mixed state with the above probability distribution. Using equation (5) and  $|\alpha_k|^2 - |\beta_k|^2 = 1$  in the expressions for  $P_n(k)$  and  $\langle N_k \rangle$ , one readily finds that

$$P_n(k) = \exp(-n\mu k) [1 - \exp(-\mu k)] \quad (6)$$

and

$$\langle N_k \rangle = [\exp(\mu k) - 1]^{-1} \quad (7)$$

where  $\mu = 4\pi s a_1^{-2} \approx 4\pi a_1^{-1} t_p$ . This is black-body radiation with a temperature at time  $t$  given by

$$T(t) \approx (4\pi k_B)^{-1} t_p^{-1} (a_1/a(t)) \quad (8)$$

where  $k_B$  is Boltzmann's constant, and the time dependence

arises because the wavelength at time  $t$  is  $(k/a(t))^{-1}$ . This unexpected result permits us to associate uniform temperature and entropy with the initially created particles, even in causally unconnected regions.

This result is consistent with the Einstein equation

$$a^{-1} d^2 a / dt^2 = -8\pi G \rho / 3 \quad (9)$$

where we have used  $p = \rho/3$ . Putting  $\rho = (\eta_a/2)\sigma T^4$ , where  $\eta_a$  is the number of different kinds of particles produced initially (counting each state with different quantum numbers (spin and charge) separately), and  $\sigma = \pi^2 k_B^4/15$  is the Stefan-Boltzmann constant, one finds from equation (9) with  $a(t) = Ct^{1/2}$  and  $a_1 = Ct_p^{1/2}$

$$T(t) = 2(45\pi)^{1/4} \eta_a^{-1/3} (4\pi k_B)^{-1} t_p^{-1} (a_1/a(t)) \quad (10)$$

at early times ( $t_p$  occurs here because  $G = t_p^{-2}$ ). This is the same as equation (8) to within a factor of  $\sim 7\eta_a^{-1/4}$ . This agreement is satisfactory, considering that equation (8) was obtained using rough, order of magnitude estimates, and that a dependence on  $\eta_a$  could appear in the expressions for  $s$  and  $a_1$  if the early behaviour of  $a$  depends on quantum effects involving the number of fundamental fields (we are using a model with a limited number of such fields).

## Consistency with observations

Finally, it is worth noting that the temperature in equation (10) (or equation (8)) can be connected to the present temperature. As the Universe expands, the original particles decay, interact, and annihilate, leaving  $\eta_b$  components of the present black-body radiation (counting each spin and charge state separately, and each fermion as 7/8 of a boson in forming the entropy below). Assuming that the entropy

$$S = \eta(t)(2/3)\sigma T^3(t)a^3(t)$$

in a comoving volume,  $a^3(t)$ , is conserved, one finds<sup>17-19</sup>

$$T_b \approx (\eta(t)/\eta_b)^{1/3} T(t) (a(t)/a_b) \quad (11)$$

where subscript  $b$  refers to the present time, and  $\eta(t)$  is the number of elementary constituents at time  $t$ . Assuming that the factors involving  $\eta$  are  $\sim 1$ , since powers like 1/3 or 1/4 are involved, one finds from equations (10) and (11) that (in c.g.s. units)

$$T_b \approx 7(4\pi k_B)^{-1} \hbar t_p^{-1} (a_1/a_b) \quad (12)$$

Denoting the earliest time at which the universe becomes matter-dominated by  $t_e$  (so that  $a \propto t^{2/3}$  for  $t > t_e$ ), one finds  $a_1/a_b \approx (3/2)^{2/3} (t_p/t_H)^{1/2} (t_e/t_H)^{1/6}$ , where  $t_H = (a(da/dt))_b^{-1} = 3t_b/2$  is the Hubble time<sup>20</sup>,  $t_H = 5.8 \times 10^{17}$  s. This gives

$$T_b \approx (t_e/t_H)^{1/6} (30 \text{ K}) \quad (13)$$

which equals 3 K when  $t_e \approx 2 \times 10^4$  yr, but is within a factor of 10 of 3 K for all possible values of  $t_e > 8$  d. To determine  $t_e$  in our model one would have to predict the baryon number density (or entropy per baryon).

Any theory consistent with Einstein's equations and leading to black-body radiation before  $t_e$  will yield equation (12) and (13), since equation (10) (with  $\eta_a$  replaced by  $\eta(t)$ ) follows from the Einstein equation (9) at a time  $t$  shortly before  $t_e$ . The particle creation process occurring near  $t_p$  leads naturally, however, to the interpretation of  $T_b$  in equation (12) as the redshifted temperature of the particles created with a temperature of the order of the Planck temperature ( $k_B^{-1} \hbar t_p \approx 10^{32}$  K). In that sense, a model of the present kind (or possibly starting with an anisotropic expansion) seems to point towards a plausible explanation of the origin of the density and entropy



of the black-body radiation. Our model is perhaps the simplest of those involving particle creation near the Planck time.

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# Abundance and membrane association of elongation factor Tu in *E. coli*

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*Elongation factor Tu is present in a fourfold excess over the amount seemingly involved in protein synthesis. It is identical to a major protein which we have found associated with the plasma membrane. The possibility of a specific role in that location is considered.*

In a recent study of envelope-associated proteins, we have described the properties of a homogeneous polypeptide of which there are 70,000 molecules per cell (5% of the total cellular protein)<sup>1</sup>. Several observations, particularly its release from the cell by osmotic shock but not by the conversion of cells to spheroplasts, suggested that this polypeptide is probably associated with the inner face of the plasma membrane *in vivo*. We could not identify the function of this protein with a molecular weight of 44,000. We now report that it is identical with elongation factor Tu (EF Tu) which through cyclic association and dissociation with another factor, EF Ts (ref. 2), is an essential component in protein synthesis (for review, see ref. 3). We demonstrate that EF Tu is present in substantial excess over EF Ts, and therefore over ribosomes<sup>4</sup>. This abundance and the suggested association with the membrane led us to ask what function EF Tu could exert in that location.

## EF Tu and the protein released from the cell by osmotic shock are identical

The relationship between the protein we have described<sup>1</sup> and EF Tu is shown by the following observations. Electrophoresis in sodium dodecyl sulphate (SDS) of authentic EF Tu, purified according to Miller and Weissbach<sup>5</sup>, and of the major protein released by osmotic shock yielded identical mobilities for the two proteins (not shown). The amino acid compositions of the two proteins are very similar<sup>1,3</sup>, and well within the variations usually observed between laboratories. The amino terminal ends of both proteins are blocked (ref. 1 and Wade *et al.*, unpublished, cited in ref. 6). We have confirmed this for authentic EF Tu by the method of Weiner *et al.*<sup>7</sup>. No aminoacyl residue modified in its  $\alpha$ -amino group was found after dansylation and hydrolysis of the undegraded polypeptide and after each of three successive cycles of Edman degradation. The spectra of the membrane-associated protein before and after extensive dialysis<sup>1</sup> are identical with the corresponding spectra of EF Tu (ref. 5). The functional identity of the released protein with EF Tu was tested by measuring both

GDP binding<sup>8</sup> and stimulation of the *in vitro* polymerisation of phenylalanine<sup>9</sup> in osmotic shock supernatants. The results (Table 1) show that the activities characteristic for EF Tu vary according to the amount of the major protein released into the shock fluid. To determine whether the two proteins are in fact the same, we prepared tryptic peptide maps of the purified membrane-associated protein and of EF Tu. The fingerprints obtained were very similar (Fig. 1a and b). Double-diffusion tests of antibodies specific to one

**Table 1** Correlation between release of EF Tu and of the major membrane-associated protein released from *E. coli* by osmotic shock\*

| Shock medium             | Phe polymerisation†<br>(pmol polymerised<br>ml <sup>-1</sup> ) | GDP binding‡<br>(nmol bound<br>ml <sup>-1</sup> ) | Amount of<br>membrane-associated<br>polypeptide§<br>(% total cell<br>protein) |
|--------------------------|----------------------------------------------------------------|---------------------------------------------------|-------------------------------------------------------------------------------|
| Water                    |                                                                |                                                   |                                                                               |
| Supernatant¶             | 74 (80%)                                                       | 4.0 (51%)                                         | 3.6 (74%)                                                                     |
| Pellet¶                  | 18 (20%)                                                       | 3.8 (49%)                                         | 1.3 (26%)                                                                     |
| 0.5 mM MgCl <sub>2</sub> |                                                                |                                                   |                                                                               |
| Supernatant¶             | 21 (25%)                                                       | 0.76 (9%)                                         | 0.4 (8%)                                                                      |
| Pellet¶                  | 62 (75%)                                                       | 7.4 (91%)                                         | 4.4 (92%)                                                                     |

\*Cells (*E. coli* K12, derivative BHB 960) were grown to a density of  $6 \times 10^8$  ml<sup>-1</sup> and osmotically shocked using the procedure of Nossal and Heppel<sup>9</sup> with H<sub>2</sub>O or 0.5 mM MgCl<sub>2</sub> as shock medium. Equal aliquots of the supernatants and pellets, taken up in the original volume and sonicated, were assayed as indicated below. Total activities were determined for all experiments by assaying sonicated, but unfractionated, cells. These were always within 10% of the sum of osmotic shock supernatants and pellets. Cell lysis was monitored by measuring  $\beta$ -galactosidase activity<sup>10</sup> in supernatants and pellets, and was always  $\leq 20\%$  with water and  $\leq 10\%$  with MgCl<sub>2</sub>.

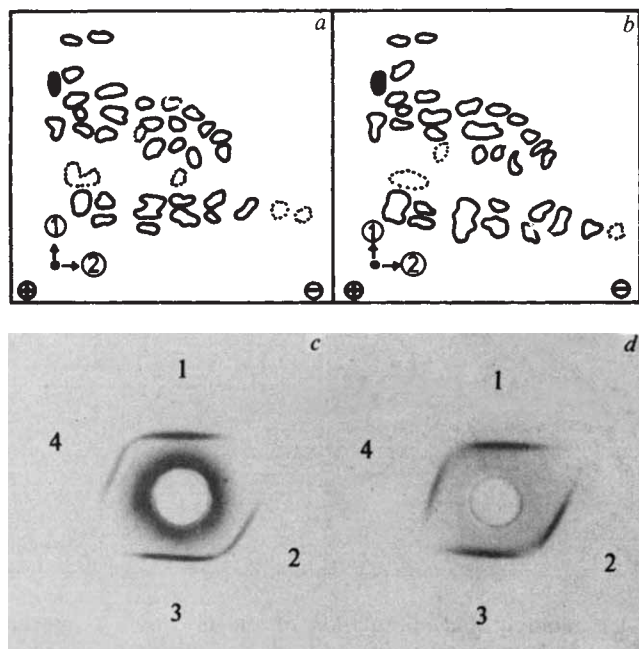
†Phenylalanine polymerisation<sup>9</sup> using <sup>14</sup>C-phenylalanine tRNA and washed ribosomes as described previously<sup>11</sup> was measured for 5-min periods at 30 °C. EF Ts (purified as described in Fig. 2) and EFG free of EF Tu activity (an S-100 of *E. coli* BE was treated at 55 °C for 15 min, ref. 12) were added in excess. Purified EF Tu polymerised 0.80 nmol Phe per 5 min per mg protein in these conditions, close to the value of 0.88 calculated from ref. 8.

‡Binding was measured with 5  $\mu$ M <sup>14</sup>C-GDP (Amersham) by filtering the reaction mixture after 15 min at 25 °C through nitrocellulose filters (Sartorius, 0.45- $\mu$ m pore size).

§Estimated by quantification of the peaks representing the membrane-associated polypeptide in densitometric scans of stained polyacrylamide slab gels, as described previously<sup>13</sup>.

¶Protein concentrations were: 0.6 and 8.5 mg ml<sup>-1</sup> in the shock fluid and the residue, respectively, in the presence of magnesium ions, and 2.5 and 7.5 mg ml<sup>-1</sup> in its absence.

||Figures in parentheses indicate the percentage of the total activity or of the total membrane-associated protein, respectively.



**Fig. 1** Comparison of osmotically released polypeptide and EF Tu. The two peptide maps represent the patterns after ninhydrin staining (a) and an autoradiogram (b) of the same chromatogram. *E. coli* BE cells were grown as described previously<sup>13</sup> and labelled with a <sup>14</sup>C-amino acid mixture at a cell density of  $10^8$  ml<sup>-1</sup>. Cells were collected at  $6 \times 10^8$  ml<sup>-1</sup>, washed and subjected to osmotic shock. Concentrated shock fluid was subjected to gel electrophoresis in SDS. The major band (44,000) was eluted, and the concentration of detergent lowered by dialysis overnight at low ionic strength. A tenfold excess of pure, unlabelled EF Tu (ref. 5) was then added. The mixture was digested with TPCK-treated trypsin and subjected first to chromatography (dimension 1) and then electrophoresis (dimension 2) on silica gel thin layer plates (Kodak 6061) with solvent systems as described previously<sup>14</sup>. The minor differences of the maps, if they are not artefactual, may be due to the fact that there are two genes coding for EF Tu (ref. 15), and that the two gene products have been enriched to different extents in the preparation procedures for EF Tu and the protein released by osmotic shock. From the number of spots (34) and the sum of arginyl and lysyl residues in the two proteins<sup>1,5</sup>, the possibility of a mixture of two non-identical proteins can be excluded. The Ouchterlony double-diffusion test shows the reaction of antibodies specific for the osmotically released protein with EF Tu (wells 1 and 3) and an osmotic shock supernatant (wells 2 and 4), (c); and the reaction of antibodies specific for EF Tu with the same samples in the outer wells (d).

protein yielded precipitin lines continuous with that of the other (Fig. 1c and d).

### Excess of EF Tu over EF Ts in the cell

It has been reported that EFs are synthesised in stoichiometric amounts with ribosomes, and that their quantities in the cell vary with the growth rate of bacteria<sup>4</sup>. In the case of EF T, this observation should be restricted to EF Ts, since the antibodies, used for the quantifying of the factors, were specific for EF Ts, and did not interact with EF Tu (ref. 16). In view of the fact that there are 70,000 molecules of EF Tu per cell<sup>1</sup>, and that we found little variation in the different growth conditions used, we re-determined the proportions of EF Tu and EF Ts in the cell.

First, we determined the amounts of the two factors in the three fractions from an ammonium sulphate precipitate, which represented an intermediate step in the purification procedure<sup>17</sup>. Table 2 shows that almost two-thirds of the total EF Tu was contained in fraction C. Activity determined in that fraction by the phenylalanine polymerisation assay, however, is considerably less<sup>17</sup>, an observation explained by the virtual absence of EF Ts in that fraction. This result was confirmed by gel filtration chromatographic

analyses of extracts A, B and C (Fig. 2). EF Tu is therefore presumably underestimated when an activity assay designed to detect the function of the complexed factor (EF T) is used, but is revealed by gel electrophoretic analyses of the various fractions.

From the data in Table 2 and Fig. 2, it can be estimated that approximately 80% of total EF Tu occurs in the non-complexed form. The ratio of EF Tu to EF Ts would be 5:1. To confirm this result, we used an independent method. The estimation of the amount of protein in the

**Table 2** Quantification of EF Tu from stained gels and EF Ts by activity assays in ammonium sulphate fractions\*

| Extract (% (NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> ) | EF Tu† (% total) | EF Ts‡ (% total) |
|--------------------------------------------------------------|------------------|------------------|
| A (27.8% eluate)                                             | 7.8              | 25               |
| B (24% eluate)                                               | 28.2             | 75               |
| C (19.6% eluate)                                             | 64.0§            | None detected    |

\*A crude extract of 100 g *E. coli* BE was carried through the ammonium sulphate fractionation step<sup>17</sup> in the purification of EF T and EF G. No EF Tu or EF T was found in the residue after the 19.6% extraction.

†Estimated from densitometric scans of stained SDS-polyacrylamide gels<sup>13</sup> of the three fractions.

‡Measured by exchange<sup>18</sup> of <sup>14</sup>C-GDP with purified EF Tu-GDP.

§Our finding of large quantities of non-complexed EF Tu is in agreement with results of Arai *et al.*<sup>19</sup>, who purified EF T, EF Tu and EF Ts by ion exchange chromatography in the presence or absence of GDP. They also found a large fraction of free EF Tu in cell extracts.

bands on SDS gels with mobilities corresponding to EF Tu and EF Ts (Fig. 3) yielded a ratio of  $3.6 \pm 0.4$  (Table 3). Although we have already determined that the band corresponding to EF Tu consists of at least 90% of this factor<sup>1</sup>, we do not know whether other proteins comigrate in the position of EF Ts; a value of 3.2 is therefore a minimal estimate. It is reasonable to conclude, therefore, that the two factors occur in a ratio of approximately 4:1, independent of the growth conditions used.

Also presented in Table 3 are the growth rates obtained for *Escherichia coli* BE cells in different media and the corresponding values for the proportion of total cell protein represented by EF Tu. Cells growing very slowly synthesised about half the amount of EF Tu as those grown in richer media. Since the ratio of the two factors remains the same (3.4), there is approximately half as much EF Ts in cells grown on the poorer medium. Although with the strain used in this study, minimal media containing glucose still yielded short generation times (Table 3), our results are consistent with the previously published variation of the level of EF T with growth rate<sup>4</sup>. The synthesis of EF Tu thus seems to be coordinated with that of ribosomes and EF Ts, but at a much higher level.

### Possible functions for EF Tu at the membrane

Since EF T occurs in an approximately equimolar ratio to ribosomes<sup>4</sup>, and this complex is known to contain EF Tu and EF Ts in stoichiometric amounts, 20–30% of the total complement of EF Tu seems to be involved in protein synthesis at any given time. The simplest explanation for the 'excess' of EF would be that the binding of aminoacyl-transfer RNA to the acceptor site in the ribosomes is inefficient, and that a large excess of the protein responsible for this process is required for effective protein synthesis. Alternatively, or in addition, EF Tu may be responsible for other functions in the cell. It is well known that when *E. coli* is infected with RNA phages such as Q $\beta$ , this protein becomes a subunit in the enzyme responsible for RNA replication<sup>23</sup>. It may further exert a control function in transcription, in conjunction with the effector



Table 3 Stoichiometry of EF Tu and EF Ts

| Method                                                                         | Medium                               | Generation time (h) | EF Tu<br>(% total protein)* | EF Tu/EF Ts<br>(mol per mol)† |
|--------------------------------------------------------------------------------|--------------------------------------|---------------------|-----------------------------|-------------------------------|
| Quantification by Sephadex G-100 chromatography of ammonium sulphate fractions | M9 (minimal)                         | 0.87                | —                           | 5.1‡                          |
| Stain quantification from gels                                                 | M9 (minimal)§                        | 0.87                | 5.1                         | 3.3                           |
|                                                                                | M9+1% casamino acids mixture (Difco) | 0.48                | 4.6                         | 4.0                           |
|                                                                                | Tryptone broth (Difco)               | 0.48                | 4.5                         | 3.8                           |
|                                                                                | Acetate (1%)                         | 4.5                 | 2.7                         | 3.4                           |
| Determination of radioactivity¶ eluted from gels                               | M9 (minimal)                         | 0.87                | —                           | 3.5                           |

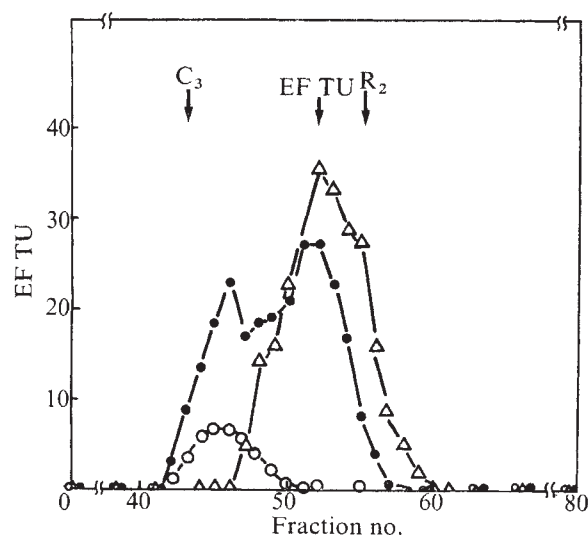
\*Calculated from densitometric scans of SDS gels (see Fig. 3).

†Bands corresponding to EF Tu and EF Ts were cut from stained gels. The stain was eluted with dimethylsulphoxide and the  $A_{600}$  of eluates measured (for details, see ref. 22). Each value is the average of at least four determinations.

‡Calculated from data given in Table 2 and the integrated peaks in Fig. 2.

§Cells were grown to densities of  $6 \times 10^8$  ml<sup>-1</sup> in the media indicated, collected, dissolved directly in SDS, and subjected to gel electrophoresis in the detergent.

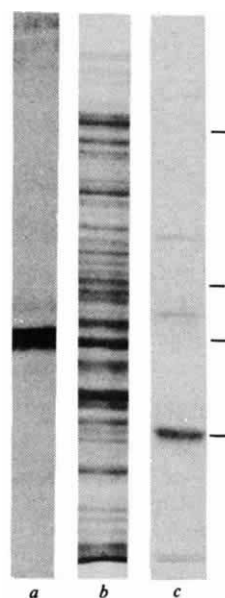
¶Cultures in M9 medium were labelled at cell densities of  $10^8$  ml<sup>-1</sup> with  $0.25 \mu\text{Ci}\cdot\text{ml}^{-1}$  U-<sup>14</sup>C-amino acid mixture (Amersham). At densities of  $6 \times 10^8$  ml<sup>-1</sup>, they were collected and electrophoresed as described above. Bands were dissolved in 30% H<sub>2</sub>O<sub>2</sub> at 60 °C overnight and counted as described previously<sup>22</sup>. The value given is the average of four determinations.



**Fig. 2** Gel filtration chromatographic analyses of the three extracts from an ammonium sulphate fractionation (see Table 2). Eluates A, B and C were dialysed into 50 mM Tris-HCl, 10 mM MgCl<sub>2</sub>, 1 mM dithiothreitol and 0.1 M KCl, and chromatographed on a calibrated Sephadex G-100 column in the same buffer. All fractions containing material absorbing at 280 nm were subjected to electrophoretic analysis in SDS. The gels were stained with Coomassie blue. From the patterns obtained, the amount of EF Tu was quantified by integration of the peaks obtained by scanning densitometry (for details, see ref. 13). Units on the ordinate are arbitrary. ○, Extract A; ●, extract B; △, extract C. C<sub>3</sub> and R<sub>2</sub> indicate the catalytic and regulatory subunits of aspartate transcarbamylase, with molecular weights of 100,000 and 34,000, respectively<sup>20</sup>. All EF Tu in extract A and 40% of it in extract B chromatographed with a distribution coefficient ( $K_d$ ) corresponding to a molecular weight of 80,000. The remaining 60% in extract B and all in extract C had a  $K_d$  identical to that of purified EF Tu (arrow). The composition of the complexed form of EF Tu (80,000) was determined by re-chromatography of the pooled fractions (43–46) in the presence of 1 mM GDP in the column buffer. In these conditions, EF Tu eluted quantitatively at the position indicated. The only other polypeptide to shift position in this second dimension was one with a mass of 32,000. The protein is present in approximately stoichiometric amounts with EF Tu. Its identity to EF Ts was confirmed by separating the two proteins which change their elution position in the presence of GDP using ion exchange chromatography<sup>5</sup>. Determination<sup>19</sup> of EF Ts activity yielded  $2.7 \times 10^5$  U mg<sup>-1</sup> in the peak containing the 32,000 polypeptide. We have used this two-dimensional procedure to purify EF Ts.

guanosine tetraphosphate<sup>24,25</sup>. It is unclear, however, whether these functions could account for the large amount of EF Tu, and for the high ratio of EF Tu to Ts.

We have shown<sup>1</sup> that 50–80% of EF Tu is released from the cell by osmotic shock (data corrected for cell lysis). We have suggested that the released fraction is associated with the inner face of the plasma membrane, suggesting that a specific function is carried out in that location. Membrane association of many processes involved in macromolecular synthesis in bacteria has been suggested<sup>26</sup>, and some of these, in turn, may be related to EF Tu (see above).



**Fig. 3** Gel electrophoretic patterns in SDS of pure EF Tu (a), whole cells directly dissolved in sample buffer<sup>21</sup> (b) and EF Ts, purified according to the two-dimensional procedure given in Fig. 2 (c). Scanning densitometry indicated that the main band of gel c constitutes 80% of the total protein applied. Determination of EF Ts activity in the GDP-exchange reaction<sup>18</sup> yielded a value 20% smaller than that found for the pure factor<sup>18</sup>. It can therefore be concluded that the major band is pure EF Ts. The growth conditions and the method for estimating the intensities of the bands corresponding to EF Tu and EF Ts in gel b are described in Table 3. Molecular weight standards indicated were: 1, phosphorylase a (95,000); 2, catalase (58,000); 3, ovalbumin (45,000); 4, C chain of aspartate transcarbamylase (33,000).



The protein may, of course, also be functional in the synthesis of proteins on membrane-bound ribosomes<sup>27</sup>. The evidence for a compartmentalisation of ribosomes in bacterial cells, however, is not fully convincing; according to recent estimates<sup>28</sup>, it would account for only one-third of the ribosomes and therefore for less than 10% of EF Tu.

One observation may be of value in determining whether EF Tu provides a link between several cellular processes in the membrane, or whether it may have other functions there. Morrissey *et al.*<sup>29</sup> have reported that the ribosomal proteins L7/12, known to interact with EF Tu on ribosomes (see ref. 30 for references), also occur in the non-ribosomal fraction of the cell in amounts similar<sup>29</sup> to those of 'excess' EF Tu. It would be interesting, therefore, to determine whether these proteins also interact with EF Tu at other cell sites, and if so, whether the function of the complex may be more amenable to analysis than that of either component alone.

Yet another possibility is suggested by our observation that the membrane-associated fraction of EF Tu is partially precipitated by calcium, and quantitatively by vinblastine ions. We have considered the possibility that these and related observations may indicate a structural role for this protein at the bacterial membrane<sup>1</sup>. Finally, Jaskunas *et al.*<sup>15</sup> have reported that at least two genes code for EF Tu. Although the products of these genes seem to be very similar<sup>15</sup>, this observation opens up the possibility of a genetic approach to investigating the functions of EF Tu in the cell.

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*Note added in proof:* Furano<sup>31</sup> has also reported a high excess of EF Tu over ribosomes. According to his interpretation, the excess of the elongation factor allows binding of the entire aminoacyl-tRNA pool in the cell.

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# Production of yeast alcohol dehydrogenase isoenzymes by selection

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*Mutants of yeast alcohol dehydrogenase have been produced that protect the cell against the poisonous aldehyde acrolein by increasing the NADH-NAD ratio. The altered properties include changes both in binding constants and in cooperativity. Such mutants may be useful in exploring the nature of adaptation at the molecular level.*

WORK in the field of molecular 'evolution' in the laboratory has so far been directed towards the selection of mutant enzymes with a high affinity towards unusual substrates<sup>1-3</sup>. I report here on a specific selective system that produces mutants of yeast alcohol dehydrogenase (ADH, EC 1.1.1.1) in which other functions have been altered. The kinetic alterations are sufficiently marked to justify the view that these mutant gene products are new isoenzymes. There is at this locus a potential for great diversity in adaptational response, which may cast some light on the fine details of the evolutionary process.

The selection procedure has been set forth in detail elsewhere<sup>4</sup>, and relies on two observations. The first is that petite yeasts (those unable to respire aerobically) synthesise only one of the two isoenzymes found in the cytoplasm. This form, ADH-I (ref. 4), is normally constitutive under laboratory conditions, has a high  $K_m$  (Michaelis-Menten constant) for ethanol, and seems to be chiefly responsible for the production of ethanol from acetaldehyde in cells grown anaerobically<sup>5</sup>. The other

cytoplasmic isoenzyme, ADH-II, is coded by an unlinked locus<sup>6</sup>, has a much lower  $K_m$  for ethanol, and has a relative mobility at pH 8.6, with ADH-I taken as 100%, of 110%. ADH-II is found only in aerobically grown grande yeast. A primary function of ADH-II is to convert ethanol accumulated during anaerobic growth to acetaldehyde, with the concomitant reduction of NAD. ADH-I can also accomplish this task, though presumably less efficiently; grande mutants (those able to respire aerobically) free of ADH-II have been produced in several laboratories<sup>4,7</sup> and grow satisfactorily on ethanol as the sole carbon source.

The second observation on which the selection scheme is based is that grande cells lacking cytoplasmic ADH activity cannot be induced to become petite. They die instead, indicating

**Table 1** Concentrations of NADH and NAD in crude extracts of wild-type and mutant cells

| Strain            | µg NADH per mg protein | µg NAD per mg protein | NADH-NAD ratio |
|-------------------|------------------------|-----------------------|----------------|
| XW517-2D (ADH-I)  | 0.035                  | 0.568                 | 0.062          |
| XW520-2A (ADH-II) | 0.152                  | 0.913                 | 0.166          |
| XW517-S-AA-1      | 0.296                  | 0.781                 | 0.379          |
| XW517-S-AA-5      | 0.196                  | 1.038                 | 0.188          |

Protein was determined by the method of Lowry *et al.*<sup>15</sup>. Cells were grown in YEPD + 2% EtOH. Average of two determinations.

that ADH activity is essential for the survival of petite cells. Reinforcing this conclusion, it has proved impossible to select petite strains to yield cells lacking ADH activity. It seems likely that glycolysis cannot proceed without the presence of ADH to catalyse the production of ethanol as a final step, although grande cells lacking ADH activity can survive by means of oxidative phosphorylation alone.

Cells lacking ADH activity and ADHs with altered kinetics were selected by an elaboration of the scheme originally used by Megnet<sup>8</sup>. Allyl alcohol,  $\text{CH}_2\text{CHCH}_2\text{OH}$ , is readily oxidised by yeast ADH to acrolein (acrylaldehyde). Acrolein is highly toxic to yeast cells, although the mechanism of its toxicity is in doubt<sup>9</sup>. Selection on a specific sequence of media with increasing allyl alcohol concentrations resulted in the production of grande yeast strains lacking both cytoplasmic and mitochondrial ADHs (ref. 3). These strains could survive in allyl alcohol concentrations up to 1.6 M, compared with 1 mM for the wild type, which was a strong indication that allyl alcohol by itself was harmless to the cell. These were the strains that could not be converted to petite, demonstrating that petites lacking ADH activity could not survive.

**Table 2** Acetaldehyde and ethanol in medium of cells grown anaerobically to log phase

| Strain           | Acetaldehyde (mM) | Ethanol (M) |
|------------------|-------------------|-------------|
| XW517-2D (ADH-I) | 1.2               | 0.096       |
| S-AA-1           | 4.84              | 0.05        |
| S-AA-5           | 1.2               | 0.12        |

Concentrations were measured enzymatically with commercial ADH (Sigma). Cells were grown in a New Brunswick Bio-Flo chemostat, model C-20, modified by the addition of a closed-circuit pump passing medium and cells from the instrument through a photocell monitor connected by a feedback loop to the chemostat's medium pump. The concentration of cells in the chemostat chamber could therefore be kept constant and at log phase. All strains were grown to a level of  $3 \times 10^7$  cells  $\text{ml}^{-1}$ , without aeration, since introduction of air rapidly removes acetaldehyde from the medium by evaporation. Thus, the pattern of excretion of the strain producing ADH-II could not be checked accurately since in anaerobic conditions it also produced only ADH-I.

The rationale for producing mutant enzymes with altered kinetics was therefore straightforward. Selection was carried out on petite strains grown on complete medium with increasing amounts of allyl alcohol. The assumption was that petite strains could not simply escape by mutating to ADH<sup>-</sup>, since they required ADH activity. Only those cells containing an enzyme that could somehow discriminate between the normal substrate, ethanol, and the potentially dangerous one, allyl alcohol, would survive. It was assumed initially that the mutant enzymes thus obtained would show marked kinetic differences between the two substrates, but this was not the case and in retrospect it can be seen to be unlikely because of the similarity between the two molecules. Instead, the mutant enzymes changed the intracellular environment so as to shift the equilibrium between allyl alcohol and acrolein in the direction of the harmless alcohol. This was done by shifting the NADH-NAD ratio.

### Selection and physiological effect of mutant enzymes

The mutants were selected from a wild-type cytoplasmic petite, XW517-2D, a mating type, by plating on YEPD (1% yeast extract, 2% Bacto-peptone, 2% dextrose, all w/v, all from Difco) to which 2% ethanol (v/v) and allyl alcohol had been added. Spontaneous mutants were selected that grew well at 20, 40 and 60 mM allyl alcohol, cloned at each stage and plated at the next highest concentration. Vigorous clones from the highest concentration of allyl alcohol were used.

Two independently derived mutants were chosen to be investigated thoroughly, XW517-S-AA-1 and XW517-S-AA-5.

Each migrated electrophoretically more slowly than the ADH-I from XW517-2D at pH 8.6, 93% and 85% respectively. Both were shown to be allelic to ADH-I and not to ADH-II by the appropriate crosses. When tetrads from crosses of the mutant to the wild type were made petite, resistance to 60 mM allyl alcohol segregated 2:2 in the tetrads and was invariably found to be associated with the mutant enzyme.

A further useful spontaneous mutation occurred in one of the segregants from a cross involving S-AA-1. This strain, which was thought to be petite, was found to produce ADH-II, and grew very slowly on glycerol as the sole carbon source. The strain had been converted to petite using ethidium bromide<sup>10</sup>, but the conversion was incomplete. This enabled us to investigate the physiological properties of ADH-II on a genetic background that was almost petite, and to compare them with the properties of the mutant enzymes and of ADH-I. This strain was designated XW520-2A.

Kinetic studies of the mutant enzymes showed that there was no pronounced change in their affinity for allyl alcohol. Indeed, the kinetic changes were slight and did not involve a large increase in  $K_m$  for this substrate. This was puzzling until we realised that neither allyl alcohol nor acrolein is likely to be metabolised further by the cell. In vivo therefore, they should eventually reach some approximation of their apparent thermodynamic equilibrium

$$K'_{eq} = \frac{[\text{Acrolein}][\text{NADH}]}{[\text{Allyl alcohol}][\text{NAD}]}$$

complicated by the possibility that substrate and product may be acted on differentially by active transport systems.

This equilibrium could not be determined precisely because of side reactions entered into by the acrolein, but it approximates that for ethanol and acetaldehyde ( $1.15 \times 10^{-4}$  at pH 7.0 (ref. 11)). Thus, an increase in the NADH-NAD ratio in the cell will produce a corresponding decrease in the acrolein-allyl alcohol ratio. Since the last compound is harmless, this should protect the cell. I therefore investigated changes in the ratio and changes in the physiology of the mutant cells.

Oxidised and reduced NAD were measured in whole-cell preparations by the Everse *et al.*<sup>12</sup> modification of the method of Greengard *et al.*<sup>13</sup> (Table 1). All three mutants, including the near-petite that produces primarily ADH-II, showed a pronounced increase of reduced relative to oxidised NAD.

Such a shift should be associated with a shift in the relative amounts of ethanol and acetaldehyde excreted by the mutant cells. The amounts of acetaldehyde and ethanol in the medium, measured enzymatically, are shown in Table 2. The two mutants show different patterns, with S-AA-5 producing more ethanol and S-AA-1 producing more acetaldehyde than the wild type. As we shall show, this was consistent with the kinetic changes.

If a shift in the NADH-NAD balance in the cell is responsible for resistance, growing the cells in conditions designed to restore the original balance should abolish the resistance, and that is what happened. It had been observed that acetaldehyde is lost rapidly from aerated but not from non-aerated cultures. The reaction should therefore be driven in the direction of ethanol in a closed system, but not in one in which the acetaldehyde is given a chance to escape. Further, addition of ethanol, even in anaerobic conditions, should drive the reaction back towards acetaldehyde, restoring the resistance. S-AA-1 and the ADH-II mutant showed the expected pattern, growing well under aeration in the presence of 20 mM allyl alcohol, but not without aeration. The resistance was readily restored in anaerobic cultures by the addition of ethanol. S-AA-5, however, showed some growth at 20 mM allyl alcohol without aeration. Ethanol also increased the resistance of this mutant. It thus seemed likely that the resistance of these mutants could be directly traceable to an enzyme-mediated shift in the proportions of reduced to oxidised pyridine nucleotides. Detailed kinetic studies bore this out, and cast some light both on the mechanisms



of the wild-type isoenzymes and on how these mechanisms have been altered in the mutants.

### Kinetics of wild-type and mutant enzymes

Table 3 gives the kinetic measurements of the ethanol-acetaldehyde reaction for the two wild-type isoenzymes and the two mutants. Allyl alcohol kinetics were also obtained, and showed differences analogous to those for ethanol, but it has so far proved impossible to obtain consistent acrolein kinetics because of side reactions. A number of kinetic parameters were obtained, as detailed in the legend to Table 3.  $K_A$  and  $K_B$  are the  $K_m$ s for substrate at infinite cofactor concentration and cofactor at infinite substrate concentration respectively;  $K_{AB}$  is the  $K_m$  for substrate and cofactor.

The kinetic data can be considered on two levels: the differences in  $K_m$  and  $V_{max}$ , and the differences in cooperativity. Both are approximately consistent with the physiological data and with the working hypothesis concerning the nature of allyl alcohol resistance.

ADH-I and ADH-II do not differ greatly in the  $V_f/V_b$  ratio, but show markedly different  $K_m$ s. ADH-II exhibits an extreme reduction in both  $K_{ABf}$  and  $K_{ABb}$ , which is what one would expect with an isoenzyme primarily concerned with oxidation of ethanol to acetaldehyde. In aerobic conditions, acetaldehyde must swiftly be converted to acetyl CoA, so the very low  $K_{ABb}$  does not normally prevent the reaction from proceeding towards acetaldehyde. The almost petite strain XW-520-2A allows accumulation of acetaldehyde and NADH, however, which explains why this strain is resistant to allyl alcohol.

S-AA-1, which excretes four times the usual amount of

acetaldehyde and loses its resistance to allyl alcohol in anaerobic conditions, shows a twofold reduction in the velocity of the back reaction coupled with a twofold reduction in the  $K_{ABb}$ . S-AA-5, which excretes more ethanol than the wild type, shows a fivefold reduction in both  $K_{ABf}$  and  $K_{ABb}$ . Although the kinetic alterations in S-AA-1 might themselves be enough to explain the shifts observed in the NADH-NAD ratio and in the pattern of excretion, they do not seem to help in the case of S-AA-5. It is also possible that some of the NADH-NAD shift has been caused by enzyme-mediated pH changes, and this is under investigation.

It is possible to obtain a rough estimate of the size and direction of cooperativity in these enzymes by calculating the ratios of  $K_{AB}$  to the product of  $K_A$  and  $K_B$ . The approximate ratios are summarised in Table 4. A ratio greater than one indicates positive cooperativity, with a greater ease of catalysis as the concentration of substrate or cofactor rises. The primary plots show an increasing  $K_m$  with decreasing substrate and decreasing cofactor concentration. A ratio less than one, on the other hand, indicates negative cooperativity<sup>14</sup>, marked by a decreasing  $K_m$  with decreasing substrate or cofactor concentration. The direction of cooperativity observed in the two wild-type isoenzymes, ADH-I and ADH-II, helps to explain their preferred direction. ADH-I shows positive cooperativity for the forward reaction, and thus at low substrate and cofactor concentrations will exhibit a reduced velocity in this direction. ADH-II shows negative cooperativity for this same reaction, and this, coupled with the greatly reduced  $K_m$ , must considerably enhance its forward reaction rate at low concentrations.

The cooperativities of the two mutants have been altered in

Table 3 Kinetic constants for the four enzymes

| Enzyme | Activity* | $V_f/V_b$ | $K_{ABf}$ (M <sup>2</sup> ) | $K_{ABb}$ (M <sup>2</sup> ) | $K'_{eq}$ (calculated) $\times 10^8$ |
|--------|-----------|-----------|-----------------------------|-----------------------------|--------------------------------------|
| ADH-I  | 352       | 0.14      | $10^{-5}$                   | $5.0 \times 10^{-7}$        | 6.8                                  |
| ADH-II | 358       | 0.215     | $1.4 \times 10^{-7}$        | $1.1 \times 10^{-9}$        | 9.4                                  |
| S-AA-1 | 296       | 0.35      | $10^{-5}$                   | $2.4 \times 10^{-7}$        | 7.2                                  |
| S-AA-5 | 79        | 0.122     | $1.8 \times 10^{-6}$        | $1.1 \times 10^{-7}$        | 7.2                                  |

| Enzyme | $K_{EtOH}$ (M)       | $K_{NAD}$ (M)        | $K_{EtOH} \cdot K_{NAD}$ (M <sup>2</sup> ) | $K_{Acet}$ (M)       | $K_{NADH}$ (M)       | $K_{Acet} \cdot K_{NADH}$ (M <sup>2</sup> ) |
|--------|----------------------|----------------------|--------------------------------------------|----------------------|----------------------|---------------------------------------------|
| ADH-I  | $2.4 \times 10^{-2}$ | $2.4 \times 10^{-4}$ | $5.8 \times 10^{-6}$                       | $3.4 \times 10^{-3}$ | $1.4 \times 10^{-4}$ | $4.7 \times 10^{-7}$                        |
| ADH-II | $2.7 \times 10^{-3}$ | $1.4 \times 10^{-4}$ | $3.8 \times 10^{-7}$                       | $4.5 \times 10^{-5}$ | $2.8 \times 10^{-5}$ | $1.3 \times 10^{-9}$                        |
| S-AA-1 | $4.2 \times 10^{-2}$ | $3.1 \times 10^{-4}$ | $1.3 \times 10^{-5}$                       | $1.1 \times 10^{-3}$ | $1.2 \times 10^{-4}$ | $1.3 \times 10^{-7}$                        |
| S-AA-5 | $2.4 \times 10^{-2}$ | $6.0 \times 10^{-5}$ | $1.4 \times 10^{-6}$                       | $2.2 \times 10^{-3}$ | $6.0 \times 10^{-5}$ | $1.3 \times 10^{-7}$                        |

\* Units  $\text{mg}^{-1}$  purified enzyme, where one unit reduced  $1 \mu\text{M}$  NAD  $\text{min}^{-1}$  at  $25^\circ\text{C}$  (pH 8.8) in the presence of  $0.3 \text{ M}$  EtOH (ref. 16).

Enzymes were purified by treatment with protamine sulphate, a heat step and ammonium sulphate fractionation. The active fraction was redissolved in  $0.01 \text{ M}$   $\text{KPO}_4$  (pH 7.0) and passed through an affinity column of DEAE-Sephadex conjugated with 8-(6-aminoethyl)-amino-NAD (ref. 17). Elution was with a pulse of NAD, and the enzyme was further purified by passage through a small DE11 column. Purity was checked by thin-layer sodium dodecyl sulphate slab acrylamide electrophoresis. Yield was typically about 80% of the activity in the crude extract. All kinetic measurements were made at pH 8.8 and  $23^\circ\text{C}$ . NAD and NADH, purchased from Sigma, were dissolved in  $0.005 \text{ M}$  phosphate buffer, pH 6.0 and 8.5 respectively, put on a  $2 \times 35\text{-cm}$  DE11 column and eluted with a  $0.005\text{--}0.2\text{-M}$  gradient of the same buffer. The NAD purification was essentially the same as that of Dalziel<sup>18</sup>; the NADH purification yielded cofactor of excellent purity with an absorbance ratio ( $A_{280}/A_{340}$ ) of only 2.25. In both purifications, other peaks were observed in the gradient. Purified cofactors were kept in the refrigerator in tightly-stoppered bottles and used within a week. No changes in their absorption properties were noted. Acetaldehyde was distilled before use and stored at  $-70^\circ\text{C}$ . The enzyme was dialysed extensively before use to remove any traces of mercaptoethanol. Kinetics were measured by monitoring the change in absorbance at  $240 \text{ nm}$  recorded at the maximum sensitivity (0.1 absorbance unit full-scale) of a Gilford 2400S spectrophotometer. Velocity readings were taken for eight different substrate and six different cofactor concentrations, each  $2/3$  the concentration of the preceding one. The reaction was measured in 3-ml cuvettes, using  $0.032 \text{ M}$  pyrophosphate buffer. It was found that zinc greatly enhanced the velocity of the back reaction (acetaldehyde to ethanol), while not affecting the forward reaction. All back reaction velocities were therefore obtained in the presence of  $5 \times 10^{-5} \text{ M}$   $\text{ZnCl}_2$ . Measurements were made in duplicate. It soon became obvious that the enzymes did not exhibit simple Lineweaver-Burk kinetics, though a Lineweaver-Burk assumption was used in the analysis. Kinetic constants were obtained by the method of Florini and Vestling<sup>19</sup>. A program written in BASIC for the Hewlett-Packard 9830 mini-computer was used to fit the eight constant-substrate lines and six constant-cofactor lines by least squares to Lineweaver-Burk plots. Then, secondary plots were derived from the inverses of the  $V$ s calculated from these primary plots to obtain  $K_A$  and  $K_B$ , the Michaelis-Menten constants for the substrate at infinite cofactor concentration and cofactor at infinite substrate concentration respectively.  $V_{max}$  for the reaction was also obtained from these secondary plots. Theoretically, it should be possible to derive  $K_{AB}$  (in  $\text{mol}^2$ ), the Michaelis-Menten constant for substrate and cofactor, since the intersection of the lines in the primary plot has the coordinates  $-K_B/K_{AB}$ ,  $(K_A - K_{AB})/K_{AB}V_{max}$ . A  $K_{AB}$  different from the product of  $K_A$  and  $K_B$  indicates the presence of interactions. The lines on the primary plot never intersected at a single point, however, showing instead a complex pattern of several intersection points. The computer was instructed to print out values of  $K_{AB}$  derived from the  $x$  and  $y$  coordinates of each of the 15 constant-cofactor line intersections and the 28 constant-substrate line intersections. The two values of each pair rarely agreed with each other, but when they did, each pair that was in agreement was also found to be close to the same value. This value was taken to be  $K_{AB}$ , and could be compared with the product of the values of  $K_A$  and  $K_B$  derived from the secondary plot. This procedure might seem arbitrary, since so much information was discarded, but fortunately a further check existed. Since kinetics for both the forward and the back reaction were available, it was possible to substitute them into the Haldane equation<sup>20</sup>  $K'_{eq} = (V_f K_{ABb})/(V_b K_{ABf})$  where  $V_f$  and  $V_b$  are the maximum velocities of the forward and back reactions respectively, and  $K_{ABf}$  and  $K_{ABb}$  the Michaelis-Menten constants for the forward and back reactions. As can be seen from the table, the  $K_{AB}$ s fit the  $K'_{eq}$  derived from equilibrium measurements<sup>11</sup> quite well, the derived value being  $7.25 \times 10^{-8}$  at pH 8.8.



different ways. In S-AA-1, positive cooperativity has been introduced into the back reaction, which has the effect of decreasing its relative rate at low concentrations. The net result, when coupled with the  $K_m$  and  $V_{max}$  changes, will be less ethanol and more acetaldehyde excreted. In S-AA-5, the positive cooperativity of the forward reaction has been reduced or lost, which should make the reaction proceed more readily at low concentrations, yet this mutant is observed to excrete relatively more ethanol than the wild type. This shift in excretion may somehow be linked to the reduced  $K_{ms}$ , but the link between the kinetic changes and the NADH-NAD shift is not as clear as in S-AA-1.

It seems likely from the kinetics that the cooperativity exists between subunits rather than between binding sites within a subunit (or the Florini and Vestling plots would all proceed through a single point), but precise values for the cooperativity

mechanism of cooperative interactions in subunit enzymes. The effect of allyl alcohol selection is to alter the kinetics of one isoenzyme towards an approximation of the kinetics of the other. In neither mutant has any improvement been noted in the velocities of the reactions; a 'better enzyme' has not been produced by these (at the most) two or three mutational steps.

Several questions of evolutionary interest can be approached using this system. Among them are: in how many ways can the molecule be altered to give kinetic changes similar to those seen in these two mutants? What fraction of the molecule has the potential to be involved in this particular adaptive change? Is there a high probability of mutational changes occurring in a particular sequence, or are there many possible sequences? And what is the distribution of amino acid substitutions bringing about these changes? One is not limited here, as is the case with the haemoglobins, to examining a sample of mutations heavily biased towards those that change the charge of the molecule: in this system, it seems highly probable that any alteration in the amino acid composition of the ADH is the result of selection and not of chance.

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**Table 4** Approximate ratios of  $K_{AB}$  to  $K_A \times K_B$  for the four enzymes

| Enzyme | $K_{ABF}/(K_{EtOH} \cdot K_{NAD})$ | $K_{ABB}/(K_{Acet} \cdot K_{NADH})$ |
|--------|------------------------------------|-------------------------------------|
| ADH-I  | 2                                  | 1                                   |
| ADH-II | 0.5                                | 1                                   |
| S-AA-1 | 1                                  | 2                                   |
| S-AA-5 | 1                                  | 1                                   |

await the completion of equilibrium dialysis studies. The situation is complicated by the fact that both substrate and cofactor seem to take part in the cooperativity. An attempt has been made to fit Hill exponents to the data, but a consistent picture has not emerged, presumably because of this complication.

It is easy to see how such kinetic alterations would lead to a shift in the reaction towards acetaldehyde, provided that there is a very efficient mechanism for removing acetaldehyde from the cell. The precise magnitude of the shift in the NADH-NAD ratio cannot be determined, since any differences between intracellular compartments are lost in our whole-cell preparations.

## Future prospects

These two mutants show specific alterations in function that can be traced to a particular selective pressure. It is likely that the present mutants and others like them can be used to probe the

# letters to nature

## Infrared profile of Milky Way at 2.4 $\mu$ m

THE diffuse component of galactic radiation has been observed in various wavelength ranges, but few observations are yet available in the near infrared range. Since the interstellar extinction is rather weak in this wavelength range, one can obtain an overview of the galactic structure, such as the distributions of infrared sources and of absorbing matter from this region of the spectrum. We have therefore attempted a balloon observation of infrared radiation at the wavelength 2.4  $\mu$ m with a band width 0.1  $\mu$ m, avoiding intense OH airglow.

The telescope comprised a silicon of 10 cm ( $F=0.8$ ) and an array of three PbS detectors attached to the focal plane, each of which had a  $3^\circ \times 3^\circ$  field of view. The whole optical

system was cooled by liquid nitrogen to reduce the background thermal radiation and improve the detector sensitivity. The absolute sensitivity was checked in flight by observing  $\alpha$  Her which was simultaneously observed at Agematsu Infrared Observatory of Kyoto University. Relative sensitivity was calibrated in flight by means of calibration light.

The Milky Way was raster scanned in the range  $23^\circ \leq l \leq 75^\circ$   $|b| < 20^\circ$ . The isophoto of the infrared surface brightness thus obtained is shown in Fig. 1. The galactic plane was found to be as much as  $6^\circ$  wide (f.w.h.m.) nearly independent of longitude. The surface brightness decreases as longitude increases, as shown in Fig. 2. The flux at the galactic equator is  $6 \times 10^{-10}$  W cm $^{-2}$   $\mu$ m $^{-1}$  at  $l=23^\circ$  and decreases to  $8 \times 10^{-11}$  W cm $^{-2}$   $\mu$ m $^{-1}$  at  $l=75^\circ$ . The longitude dependence of the infrared brightness also shows humps in the tangential directions

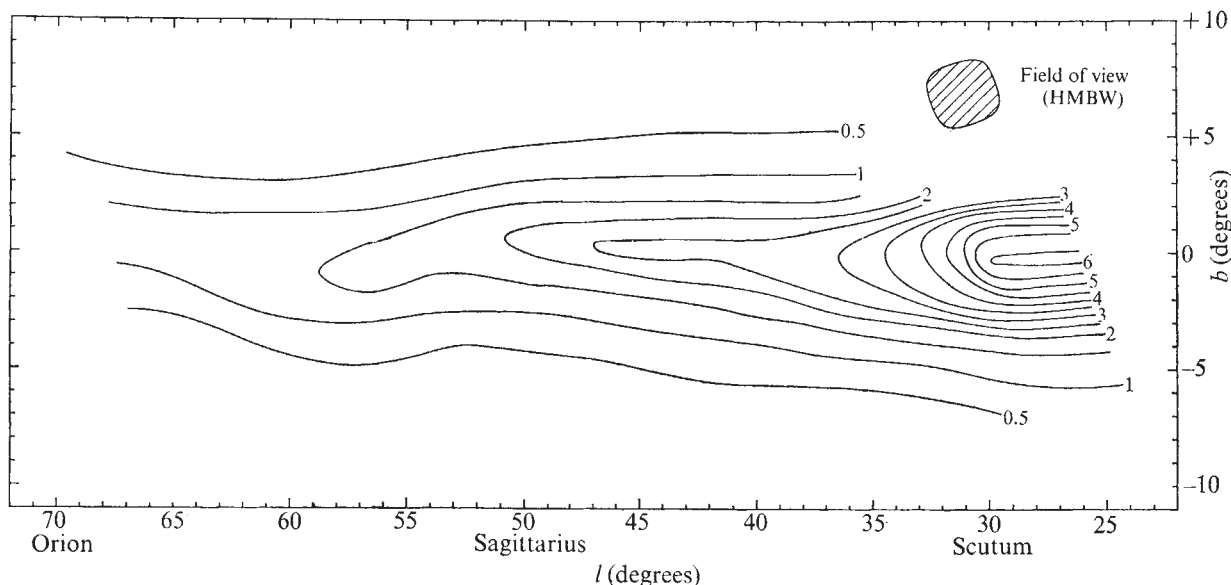
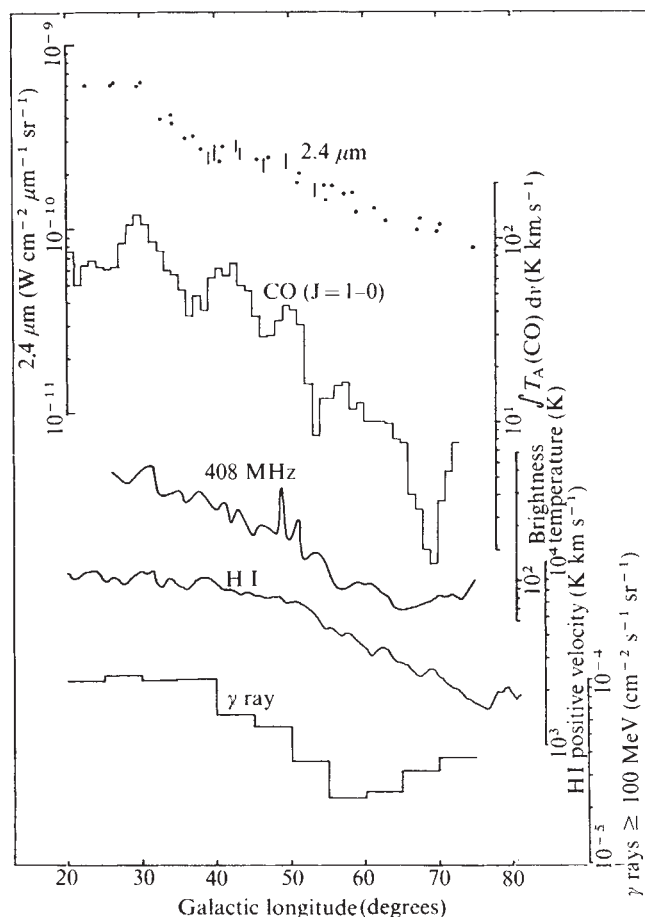


Fig. 1 Isophoto of the 2.4- $\mu$ m infrared surface brightness. The lines are drawn in steps of  $0.5 \times 10^{-10} \text{ W cm}^{-2} \text{ sr}^{-1} \mu\text{m}^{-1}$ .

of the spiral arms,  $l \approx 30^\circ, 50^\circ$ . This implies that some fraction of infrared sources are associated with particular objects in the spiral arms.

As a class of such objects, the 2.6-mm line intensity of CO

Fig. 2 Longitude dependences at the galactic equator of the 2.4- $\mu$ m intensity ( $\text{W cm}^{-2} \text{ sr}^{-1} \mu\text{m}^{-1}$ ), the 2.6-mm CO line antenna temperature integrated over velocity ( $\text{K km s}^{-1}$ ), the 408-MHz brightness temperature ( $\text{K}$ ), the 21-cm brightness temperature integrated over positive velocity ( $\text{K km s}^{-1}$ ), and the 100-MeV  $\gamma$ -ray intensity ( $\text{photons cm}^{-2} \text{ s}^{-1} \text{ sr}^{-1}$ ).



molecules<sup>1</sup> is given in Fig. 2 for comparison. Its longitude distribution shows several peaks at the positions of the infrared humps. Since the CO line intensity represents the surface density of dense molecular clouds, the association of infrared sources with the dense clouds is suggestive. The less steep longitude dependence of the infrared brightness indicates that a considerable fraction of infrared sources is distributed over the Galaxy in a similar manner to normal stars.

There may be several other objects which consist of a concentrated component and a diffuse component, the latter showing a distribution similar to general galactic matter. Of those showing longitude dependences similar to the infrared brightness over a considerable longitude range, the intensity of the radio continuum is the most remarkable example. The brightness temperature at 408 MHz (ref. 2) is also shown in Fig. 2. (The brightness temperature at 820 MHz (ref. 3) shows a similar behaviour.) The two spikes seen at  $l \approx 50^\circ$  at 408 MHz would have been too sharp to be resolved with our infrared detector. Since the radio continuum is mainly of thermal origin, this correlation indicates an H II region source. In fact, the surface density of H II regions<sup>4</sup> does correlate qualitatively with the infrared brightness.

In contrast to the distribution of H II regions, that of hydrogen atoms shows a much shallower longitude dependence. If, however, only hydrogen atoms with positive velocities are taken into account, the longitude dependence fits exactly with that of the infrared brightness for  $l \geq 40^\circ$ . The brightness temperature of the 21-cm line integrated over positive velocity<sup>5</sup> is given in Fig. 2. This would suggest that the infrared radiation observed at 2.4  $\mu$ m is emitted mainly from the region inside the solar circle. A deviation seen for  $l < 40^\circ$  may arise partly from the large optical depth ( $\sim 1$  in the low longitude region) so that the column density is not proportional to the brightness temperature.

Finally, comparison is made with the intensity of 100-MeV  $\gamma$  rays<sup>6</sup>. Since the  $\gamma$  rays are produced by collisions of cosmic rays with interstellar matter, their intensity is proportional to the product of densities of cosmic rays and interstellar matter integrated over the line of sight. Thus the distribution is correlated in part with the 21-cm line intensity and also with the density of cosmic ray sources.

It is interesting to see that the longitude dependences observed for the 2.4- $\mu$ m radiation, the 2.6-mm CO line, H II regions, the 21-cm hydrogen line of positive velocities and the 100-MeV  $\gamma$  rays are, at least in part, correlated with each other. Their interrelationships have been discussed, for example, in con-



nection with the 5-kpc ring, and thus infrared emission provides another means of investigating galactic structure.

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## Erosion and the rocks of Venus

THE remarkable photographs of the surface of Venus returned by the Venera 9 and 10 spacecraft have revealed the presence, in two different sites, of a variety of rocklike forms, some angular and some smooth. Press reports<sup>1</sup> express surprise at the absence of very efficient erosional mechanisms. It may be useful to point out instead that it is the presence, not the absence, of erosional mechanisms on Venus which is surprising. The degree of erosion of surface rocks on Venus is determined by an equilibrium between the rate of production and the rate of destruction of surface rocks. The principal causes of erosion of terrestrial rocks—running water, diurnal and seasonal temperature changes, particularly in deserts, and aeolian abrasion—are all absent on Venus. The surface temperature of 750 K is above the critical point temperature of water. Ground based radio-astronomical measurements, a comparison of the temperatures measured by Venera spacecraft at a variety of solar zenith angles, together with the high heat capacity of the massive Venus atmosphere, all clearly demonstrate that the diurnal temperature differences are a few degrees K at most<sup>2,3</sup>. The obliquity of the rotation axis of Venus is so small that there are effectively no seasons on the planet. The efficiency of aeolian abrasion depends on the velocity to a power  $\geq 3$ ; since both theory and observation show the velocities in the lower atmosphere of Venus to be about an order of magnitude less than at comparable regions in the Earth's atmosphere, it follows that sandblasting on Venus is at most  $10^{-3}$  as efficient as on Earth<sup>4</sup>.

The problem is to find a suitable source of erosion of surface rocks—a problem somewhat similar to that raised by the radar discovery of large, presumably impact, basins which, when compared with their lunar, martian and mercurian equivalents, are remarkably shallow. Two mechanisms for the erosion of crater ramparts on the surface of Venus can be suggested<sup>5</sup>; I propose that they may also be important for the erosion of the rocks photographed by Venera 9 and 10. The atmosphere of Venus contains hydrochloric acid at a mixing ratio of  $\sim 10^{-6}$ , hydrofluoric acid at  $\sim 10^{-8}$  and sulphuric acid at larger mixing ratios which, however, are as yet undetermined for the lower atmosphere of the planet<sup>6</sup>. Chemical weathering for lengthy periods by such a mixture of strong acids, even in very dilute concentrations, may be quite adequate to erode angular projections of siliceous rocks.

A second possibility arises from the high surface temperature of Venus. Although these temperatures are not sufficiently high to melt silicates, they are high enough to

bring many rather common geochemical materials (for example, NaOH, KOH, HgS and KNO<sub>3</sub>) near or to their melting points. If the rocks of Venus are comprised of such materials, even in abundances of a few tenths of a percent, the rheological properties of their low melting point components may, over long periods, be adequate to soften the contours of surface rocks. Deformation of the walls of large basins will occur over long periods, even if the lowest melting point of abundant constituents is considerably above 750 K.

With typical terrestrial values of the subsurface temperature gradient, the high surface temperature of Venus implies that the melting points of silicates should be reached a few tens of kilometres subsurface. The 'granitic' values of the uranium–potassium–thorium radioisotope ratios<sup>6</sup>, as determined by Venera 8, suggest that a terrestrial value to the subsurface temperature gradient may be a good first approximation. In this case, access of magmatic material from the interior of Venus to its surface should be considerably easier than on Earth and significant fractions of the surface may be frozen lava fields, having reached thermal equilibrium at the low temperature of 750 K. This may provide both a source of rocks too young to have been eroded and a means of filling large craters and impact basins.

Venera 9 and 10 are the first spacecraft to obtain *in situ* photographs of the surface of another planet and photographs which they have obtained open a new field of very high resolution comparative planetology.

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## Airborne ultraviolet studies of Venus

BECAUSE of the high atmospheric extinction in the ultraviolet at ground level, an airborne platform offers distinct advantage for planetary optical measurements in the wavelength region below 3,500 Å. The joint NASA–ESO Space Shuttle simulation programme (ASSESS) provided an opportunity for conducting such measurements aboard NASA's Convair 990 jet aircraft, at an altitude of  $\sim 40,000$  feet. The following summary of these measurements is a supplement to the published report on the ASSESS experiments<sup>1</sup>.

The experimental setup for airborne planetary ultraviolet studies consisted of a two-dimensional gyro-stabilised plane mirror (a heliostat) placed near a quartz side window in the aircraft. Objects outside the aircraft could be viewed at elevations between 10° and 40°. A 14" diameter Cassegrain telescope was fitted with an image position analyser star tracker which fed electrical signals to the movable secondary mirror in the telescope for stabilisation of rapid changes in image position and also to the heliostat to compensate for slow changes that were not compensated for by the gyros of the heliostat. Once an object was acquired, this system automatically tracked it within the elevation and azimuth ranges through which the heliostat could be driven to look at an object through the aircraft window.

Our primary objective was to observe Venus in the ultraviolet region. The telescope image size diameter was  $\sim 0.5$  mm. A quartz lens magnified this to a 5-mm disk in the slit plane of a 1-m Ebert–Fastie spectrophotometer.

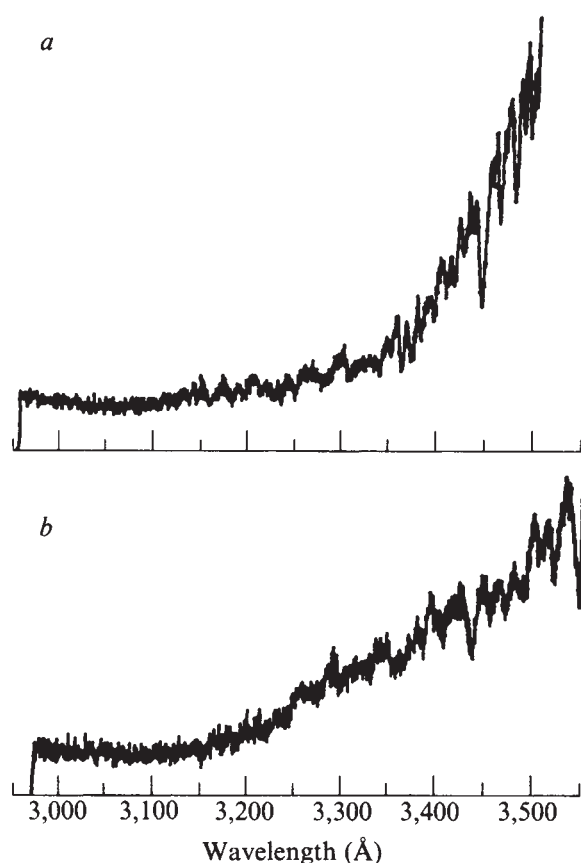


Fig. 1 Solar (a) and Venus (b) ultraviolet spectra between 2,970 and 3,540 Å at 4 Å resolution.

The primary wavelength region monitored was between 2,950 and 3,350 Å in the third order, with a resolution of  $\sim 4$  Å. A cooled S20 photomultiplier tube fitted with a quartz window, and operating in pulse counting mode, was used as a detector. A mini-computer, a digital multiplexer, a digital tape recorder and an interactive graphic system permitted data organisation for real time analysis and display of the results on a scope and a chart recorder, as well as for magnetic tape recording for subsequent detailed analysis. A D/A converter provided independent records of raw data on both chart and analog magnetic tape recorders. The entire optical and electronic system was adequately automated for single operator control in keeping with the guidelines of the shuttle simulation mission requirements.

Measurements of the solar radiation reflected from Venus were obtained on five flights at elevation angles of  $15^\circ$ – $30^\circ$ . An hour and a half of data were acquired by the experiment operator during the confined portion of the mission and two 0.5-h periods were acquired by the principal investigators later in the programme. Direct solar and lunar spectra were obtained for the same elevation angles and wavelength resolution during two of the flights. Figure 1a and b illustrates typical solar and Venus spectra. Further analysis requires normalisation of these spectra at one wavelength before obtaining the difference spectrum or ratio spectrum to remove the solar Fraunhofer and terrestrial  $O_3$  absorption features from the Venus spectrum. In a previous set of Venus observations from another expedition using the NASA Convair 990, some absorption features appeared in the wavelength regions of the  $SO_2$  bands. We will analyse our current observations, which are at higher wavelength resolution, to look for conclusive evidence of these bands as well as other features, such as the NH band at 3,357 Å, which might be present.

In addition to these Venus data, we acquired sufficient information to deduce the total  $O_3$  column densities in the Earth's atmosphere. We also monitored the ultraviolet spectra of Jupiter and terrestrial airglow  $O_2$  Herzberg and OH Meinel emissions. In summary, these flights have produced much scientific data which will be helpful to our understanding of the composition of planetary atmospheres. In addition, the evaluation of the simulated shuttle mission should be useful in the planning of scientific missions on board the space lab-shuttle system.

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## Glaciations and dense interstellar clouds

McCREA has revived the idea that the Earth's ice ages may have been caused by the interaction of the Sun with dense interstellar clouds<sup>1</sup>. In this theory, originally formulated by Hoyle and Lyttleton<sup>2</sup>, the solar luminosity is temporarily increased by the accretion of gas on to the Sun when the Solar System passes through a dense interstellar cloud. The increased insolation is assumed to cause an increase in precipitation and ice accumulation. McCrea suggests that the recurrence and duration of ice epochs can be attributed to the passage of the Solar System through compression lanes in the spiral arms. Relative velocities between the Solar System and the clouds range from 5–20 km s<sup>-1</sup>, but cloud densities of  $10^5$ – $10^7$  hydrogen molecules per cm<sup>3</sup> are called for by the model. A study of the character and grain size distribution of texturally mature lunar soils supports this model<sup>3</sup>. Here we examine several consequences of the passage of a dense cloud through the Solar System and find severe problems for this glaciation mechanism.

Since the most recent glaciation ended only  $\sim 10^4$  yr ago, we expect to find a dense interstellar cloud at a distance  $\sim 1/5$  pc, and in what follows we assume the minimum possible density for the cloud ( $\sim 3.3 \times 10^{-19}$  g cm<sup>-3</sup>) unless otherwise stated. The duration of the last glaciation was  $\geq 7 \times 10^4$  yr, indicating that the cloud dimensions must be  $\geq 1$  pc. Regardless of the relative velocity of the cloud, the solid angle subtended must be nearly  $2\pi$  steradians, or almost half the sky. Hoyle and Lyttleton acknowledged that their model implied the existence of such a dense nearby cloud, but this could not be ruled out with the observations available at that time (1939).

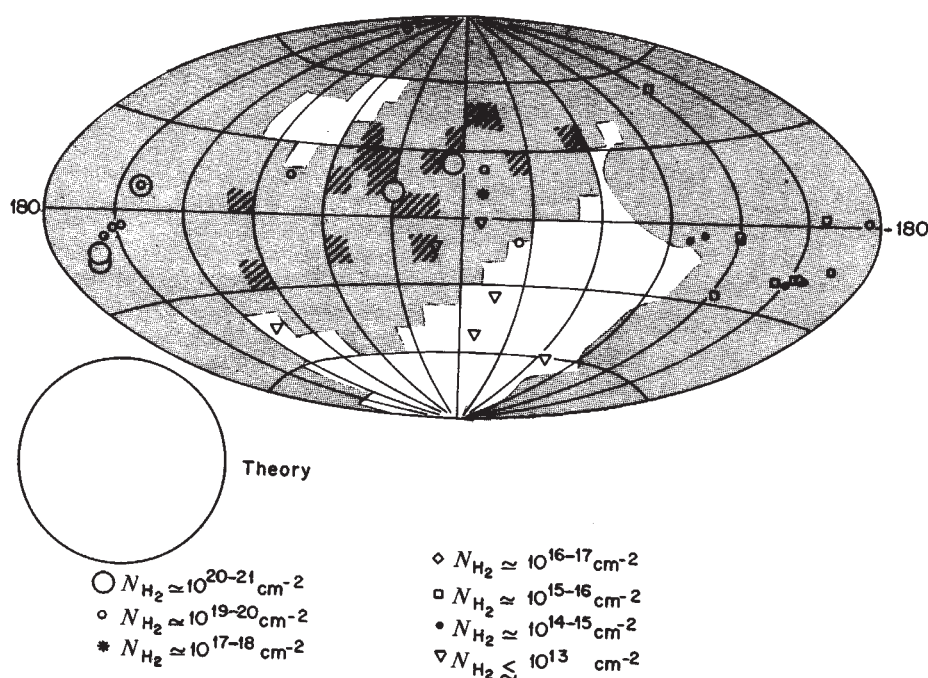
It is now straightforward to eliminate the possibility of the cloud being atomic or ionised hydrogen. The optical depth in the 21-cm line of atomic hydrogen is  $[n/(4 \times 10^{18} T_e)] \Delta X$ , where  $\Delta X$  is the cloud thickness,  $n$  is the number density of hydrogen atoms, and  $T_e$  is the spin temperature. For a reasonable value of the spin temperature of 100 K, we obtain  $\tau_{HI} \geq 750$ . If the cloud is completely ionised, then the optical depth from free-free transitions is

$$9.8 \times 10^{-21} \frac{n^2 \Delta X}{v^2 T_e^{3/2}} \ln \left[ 49.5 \times 10^{-3} \frac{T_e^{3/2}}{v} \right]$$

where  $v$  is the frequency in GHz, and  $T_e$  is the electron temperature<sup>4</sup>. For  $T_e \sim 10^4$  K and  $v = 1$  GHz, we have  $\tau_{HI} \geq 2,000$ . Clearly, no significant fraction of the cloud mass can be in the form of atomic or ionised hydrogen.

The usual possibility referred to<sup>1,2</sup> is that the cloud consists





**Fig. 1** Equal area projection in galactic coordinates of collected observations of the soft X-ray background, and column densities of  $H_2$  obtained from stellar ultraviolet absorption spectra. The soft X-ray background has been observed in shaded regions, with upper limits obtained in hatched regions. X-ray observations are not available in the clear areas. Symbols show column densities of  $H_2$ , and for those cases in which  $N_{H_2} > 10^{19} \text{ cm}^{-2}$ , the areas of the circles are proportional to the column densities. The column density predicted by the model for approximately half of the sky is illustrated by the large circle.

of molecular hydrogen. Indeed, an  $H_2$  cloud of such density would not require a significant proportion of dust for shielding since it would primarily shield itself against ambient ultraviolet radiation<sup>5</sup>. In addition, the postulated cloud is completely optically thick to X rays with energy  $\lesssim 3 \text{ keV}$  (ref. 6). In Fig. 1 we have combined published observations at 0.28 keV of the soft X-ray background<sup>7-9</sup>. At this energy the optical depth of the cloud is  $\gtrsim 1,300$ . Superimposed on these observations we depict column densities of interstellar  $H_2$ , derived from satellite observations of  $H_2$  absorption lines in the ultraviolet spectra of selected stars<sup>10,11</sup>. The largest column density reported from the ultraviolet measurements is  $N_{H_2} \approx 4 \times 10^{20} \text{ cm}^{-2}$ , whereas the model calls for  $N_{H_2} \geq 2 \times 10^{22} \text{ cm}^{-2}$ , with  $N_{H_2} \approx 4 \times 10^{23} \text{ cm}^{-2}$  being a more typical value. From the lack of extensive absorption of the soft X-ray background, and the lack of sufficient  $H_2$  column densities, we can rule out the existence of such a dense molecular cloud of large angular size.

The observational constraint that the cloud be transparent to visible light implies that for a cloud of solid particles the mean free path for mutual collisions is comparable with, or greater than, the cloud dimensions. Such a cloud cannot be appreciably accreted on to the Sun since mutual collisions are required to destroy the systematic velocity of the cloud material as it sweeps behind the Sun. Thus, the proposed cloud could not consist of dust.

The transparency to visible light also implies that, for micron-sized dust grains, the dust-gas mass ratio  $< 10^{-4}$ . This is at least 2 orders of magnitude lower than the usual value.

We now consider the dissipation of the orbits of small particles in the Solar System, because of the relative motion between the particles and the cloud. Neglecting the initial velocity of the cloud relative to the Solar System, this force will significantly disrupt the orbits on a timescale:

$$t \approx l(\rho/\rho_c)(a/GM_\odot)^{1/2}$$

where  $l$  is the radius of a particle,  $\rho$  is the mass density of a particle,  $\rho_c$  is the cloud density, and  $a$  is the orbital radius. For the zodiacal dust cloud we take  $a \approx 1 \text{ AU}$ , and  $l \approx 1 \mu\text{m}$ , and

obtain  $t \approx 4 \text{ yr}$ —even centimetre-sized particles would have been removed from the Solar System during the last glaciation. At present, much weaker dissipation mechanisms are operative, and the replenishment of the zodiacal dust cloud requires  $\gtrsim 10^5 \text{ yr}$  (ref. 8) as against the  $\sim 10^4 \text{ yr}$  since the last glaciation.

The possibility of replenishment by the dense cloud itself cannot be dismissed. Any such consideration must, however, account for the formation of the planar distribution of the zodiacal dust since the last ice age and the remarkable coincidence of the plane with the ecliptic.

We also find that this same dissipative force can remove particles of  $\sim 1 \text{ cm}$  from Saturn's rings during the  $\sim 7 \times 10^4 \text{ yr}$  glaciation period. Pollack *et al.*<sup>9</sup> interpret existing observations of the rings to indicate a mean particle radius of  $\sim 1 \text{ cm}$ . They hypothesise that small particles are continually created through meteoroid impacts. Particles considerably smaller than  $1 \text{ cm}$  are quickly removed by the Poynting–Robertson drag.

The McCrea model links purportedly periodic glacial phenomena with regular astronomical occurrences. The constraints discussed present grave difficulties in explaining at least the last glaciation in this way. These constraints, as pointed out by McCrea, are of interest since the Solar System must occasionally pass through interstellar clouds.

Another possibility is that this mechanism acts merely as a rapid trigger for a longer lived glacial episode. This tends to complicate the conjecture regarding the climatic phenomenon somewhat. The observational data constrain the angular size of the cloud  $\lesssim 30^\circ$ , indicating that the ice must persist for  $\gtrsim 5$  times the trigger duration. In this case, the optical depth of the cloud, though still severe, is reduced by a factor of five. X-ray observations in those directions not yet examined will probably restrict the possibilities even further. Although the smaller cloud would wipe out the zodiacal dust in  $\sim 20 \text{ yr}$ , the zodiacal dust would have had a longer time for replenishment. The  $6 \times 10^4 \text{ yr}$  since its passage is a sizeable fraction of the replenishment time.

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## Proterozoic oceanic crust at Bou Azzer

THE precise nature of the last great Precambrian orogeny (~600 Myr BP) is the subject of controversy, and views diverge as to whether it was a collision orogeny<sup>1-3</sup> or whether *in situ* deformation of an intracratonic orogenic belt occurred<sup>4-6</sup>. Geological evidence is given here, indicating the former presence of an oceanic domain along the northern margin of the West African Craton.

The Precambrian basement of the Anti-Atlas comprises two major structural units: the northern border of the West African Craton to the south-west, and a portion of the Pan-African Orogenic Belt to the north-east (Fig. 1). The linear Bou Azzer Complex<sup>7</sup>, composed of ultrabasic to acid, plutonic and volcanic rocks in a greenschist metamorphic environment, is located along the edge of the craton in the central part of the Anti-Atlas. Younger than the craton, which stabilised around 1,800 Myr BP, it was affected by Pan-African metamorphism (~640 Myr BP).

A vertical section, 4-5 km thick, the Bou Azzer Complex comprises, in ascending order: upper mantle peridotites

(harzburgites, dunite with some diallagites), ultrabasic cumulates (dunite-werhlite-clinopyroxenolite sequences), basic cumulates (layered gabbros), large stocks of quartz diorite, submarine lavas (diabases and spilites often displaying pillow structures) overlain by spilites, keratophyres, quartz keratophyres, and tuffs accompanied by greywackes, siltstones, limestones and jaspilites (Fig. 2). All the ultrabasic rocks have been serpentinised, in several stages; so the contact between residual mantle peridotites and overlying ultrabasic cumulates is not clearly defined. Ultrabasic and basic cumulates are the result of evolution in a magmatic chamber under a roof of tholeiitic basic lavas, in a shallow and hydrated environment undergoing distension, as shown in the layered gabbros by presence of magmatic breccias, diabases, and keratophyre sills in the lower part, which fed dyke swarms at a higher level. At the top of the layered gabbros, beneath the tholeiitic lavas, intrusion breccias comprising angular fragments of diabase enclosed in leucogabbro occur. Most of the quartz-diorite stocks, displaying flaser structures, are located between the gabbros and the overlying basic lavas. Late quartz diorites have been intruded syntectonically into keratophyres, with resulting contact metamorphism.

The Bou Azzer Complex is a typical ophiolite<sup>8</sup> assemblage displaying the same rocks facies, the same vertical sequence, and the same thickness as those of the classical Palaeozoic (Ballantrae, Newfoundland) and Mesozoic (Oman, Troodos, Vourinos) suites. In accordance with current concepts of ophiolite as oceanic lithosphere<sup>9-11</sup>, the Bou Azzer Complex is here interpreted as a sheet of allocthonous, Proterozoic oceanic lithosphere.

During the major phase of folding of the Pan-African Orogeny, which produced recumbent isoclinal folds towards the WSW and greenschist metamorphism, two palaeogeographical domains collided. The oceanic crust (ophiolite) was thrust, locally in an inverted position, over the northern border of the West African Craton, itself characterised by epicontinental limestones, quartzites and basic lavas with alkaline trend.

Also in the Pan-African Belt, 2,000 km from the Anti-Atlas, on the eastern edge of the West African Craton, in the Adrar of Iforas (Mali), disturbed ophiolite (upper mantle metaperidotites, gabbros, basic lavas, keratophyres, tuffs, jaspilites) are obducted into an epicontinental sedimentary unit.

The discovery along the margins of the West African Craton of typical ophiolites, interpreted as vestiges of Middle or Upper Proterozoic spreading oceanic crust, shows that a model based on plate tectonics, not necessarily of

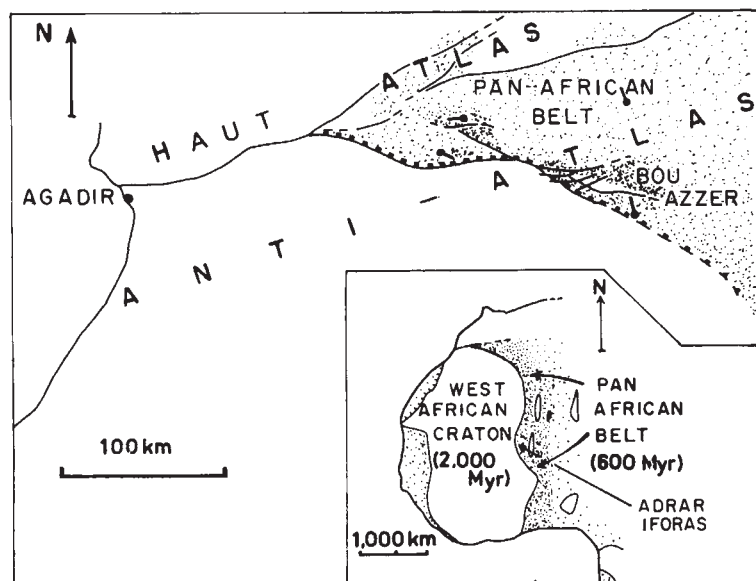
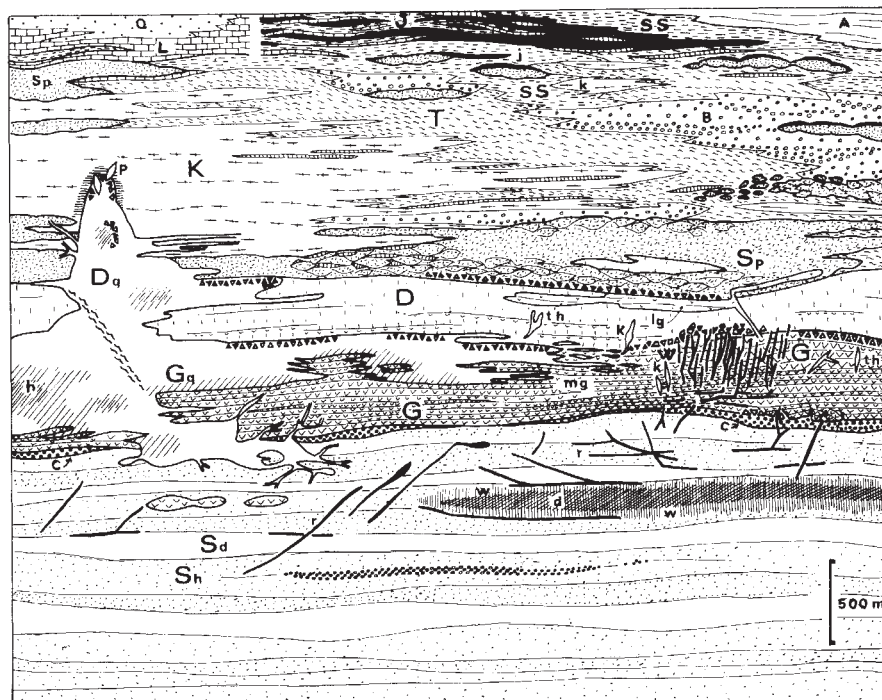


Fig. 1 West African Craton and Pan-African Belt, north-western Africa. Broken line, northern edge of West African Craton.



Fig. 2 Schematic section of the Bou Azzer ophiolitic complex.  $S_n$ ,  $S_d$ , Serpentinised harzburgites and dunites with (d, w) level of diallagite and wehrlite and (r) dykes of rodingitised gabbros; C, ultrabasic cumulates; G, layered gabbros; W, swarms of basic dykes; th, dykes of trondhjemite; mg, microgabbros; d, diabases;  $D_q$ , quartz diorites;  $G_q$ , quartz gabbros; h, hornblendites; p, intrusive apex; Sp, spilites; K, keratophyres; T, tuffs; j, jaspers; B, greywackes; SS, siltstones; L, limestones; J, jaspilites; Q, sandstones; A, arkoses.



large scale, can be applied to the Pan-African Orogenic Belt.

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## Preparation and properties of rubber dislocations

THE purpose of this note is to describe the construction and properties of a Volterra<sup>1</sup> edge dislocation made from two sheets of smooth rubber. A simple theoretical analysis illustrates the key features of the model and illuminates its interesting and unexpected behaviour.

A smooth transparent rubber sheet was manufactured by cross linking ethylene-propylene copolymer against a glass plate in a heated press<sup>2,3</sup>. After cutting the rubber into strips and removing it from the glass, two smooth pieces were brought into contact to form a laminate containing a single reversible adhesive interface (Fig. 1A). The Volterra dislocation of Burgers vector  $b$  was then formed by peeling one end of the laminate, displacing it rigidly, and re-adhering at the interface (Fig. 1B)<sup>4</sup>. Clearly, dislocations can exist in an essentially amorphous material<sup>5,6</sup>.

Viewed in the geometry of Fig. 1B the vital features of the dislocation are obvious. The dislocation is composed of two, opposite facing cracks, each capable of moving reversibly along a special weak plane in the body. These cracks differ from conventional cracks in that they can heal, where the surfaces come close together, to produce an interface comparable in

strength with that before fracture. Other close similarities between cracks and dislocations have been noted<sup>7,8</sup>.

I now consider the condition for the existence of the dislocation. The dislocation persists in spite of the attractive surface forces continually striving to close the cracks and collapse the structure. These attractions are counteracted by elastic forces in the bent rubber. In equilibrium, the dislocation reaches a length  $z$  which can be calculated using a simple energy argument.

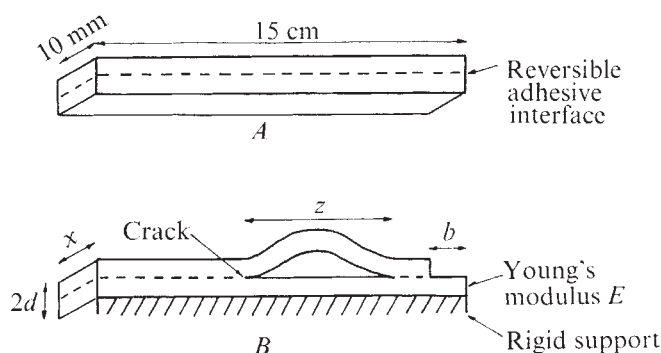
The free energy of the rubber surfaces is  $zRx$  where  $R$ , the interfacial energy, is the work needed to separate unit area of the interface. The elastic energy in the bent strip, treated as a strut collapsing at constant force<sup>9</sup>, is approximately  $\pi^2 Exd^3b/3z^2$  using the nomenclature of Fig. 1. Therefore, by energy conservation, neglecting crack tip strains which are assumed to remain constant

$$\frac{d}{dz} \left( zRx + \frac{\pi^2 Exd^3b}{3z^2} \right) = 0 \quad (1)$$

$$z = \left( \frac{2\pi^2 Ed^3b}{3R} \right)^{1/3} \quad (2)$$

This equation, giving the size of the hole in a Volterra dislocation, bears comparison with that due to Frank<sup>10</sup> who suggested that such holes should be small. Peierls<sup>11</sup> also suggested that

Fig. 1 a, Rubber laminate with a single reversible adhesive interface; b, Volterra edge dislocation in the rubber laminate.



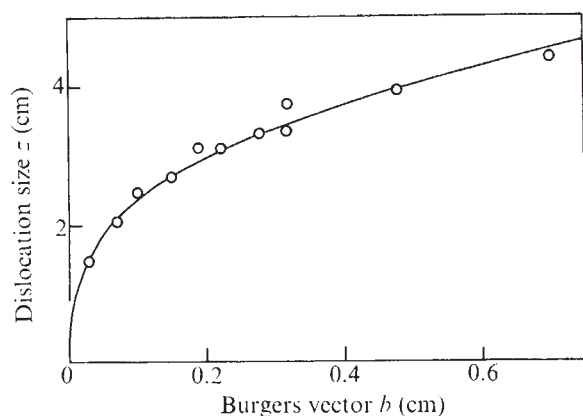


Fig. 2 The size of a rubber dislocation.—, Equation (2) with  $R = 0.57 \text{ J m}^{-2}$ .

dislocations should be small using the enigmatic argument, "... we were not actually justified in our fundamental assumptions, which took the extension of the dislocation to be large. But the dislocation cannot, in any case, be much larger than we have found, since the equations ought to be correct if it were."

The dislocations of my experiments had big holes, as shown by the results of Fig. 2. To obtain these results, a dislocation of desired Burgers vector was partially closed by applying pressure at the cracks. These cracks opened up spontaneously on releasing the pressure however, and the dimension  $z$  was measured after 10 min. Equation (2) describes the results reasonably well if the interface energy  $R$  is taken to be  $0.57 \text{ J m}^{-2}$ , about that measured in peeling tests at similar crack speeds.

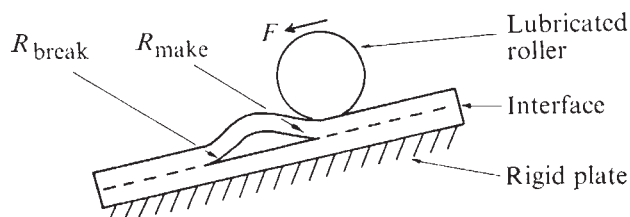
The Volterra dislocation could also be moved along the interface using a talc-lubricated roller (Fig. 3). The crack at the front of the dislocation then opened continuously and that at the rear closed. A propagation criterion was derived by considering the work done by the applied force  $F$  against the surface forces at the crack tips. If the energy expended in cracking is  $R_{\text{break}}$  per unit area, and the energy gained in reforming the interface is  $R_{\text{make}}$ , then

$$F = \alpha(R_{\text{break}} - R_{\text{make}}) \quad (3)$$

This equation, supported by experiment, shows that resistance to dislocation motion arises by interfacial hysteresis<sup>12</sup>, akin to contact angle hysteresis, where energy is dissipated in breaking and remaking an interfacial bond. A similar equation describes the rolling friction of a cylinder on a smooth surface<sup>13,14</sup>.

A stimulating phenomenon observed in these experiments was dislocation disappearance. The dislocation became shorter as it travelled, finally disappearing altogether. This was seen to be a result of the shedding of strain energy. Rubber deposited behind the moving dislocation was not unstrained, but was under slight compression. Thus the moving dislocation left some of its strain energy behind, gradually disappearing in the process. Another way of making the dislocation disappear was to deform the laminate so as to bring the sheets of rubber together across the dislocation. Stretching or bending the

Fig. 3 Dislocation movement.



laminate did this; the dislocation healed completely and was reluctant to reappear.

Equations (2) and (3) gave a rather oversimplified view of the behaviour of the dislocation. The surface energy of the rubber was not a constant but varied over several orders of magnitude with crack speed (both making and breaking)<sup>12</sup>, with temperature, and with time of interfacial contact. Equilibrium could not, in fact, be achieved because of adhesive hysteresis<sup>13</sup>.

Such non-equilibrium effects have not been taken into account in previous dislocation theories, yet they produce some amusing demonstrations. For example, the dislocation travelled at constant speed under steady load but increased in speed and became shorter as the driving force was raised; the dislocation moved faster as the temperature was increased; a second dislocation moved faster than the first; two similar dislocations repelled each other and resisted merging. If sufficient force was applied however, the dislocations joined to form a large dislocation.

These observations may be of value in explaining the deformation of materials, both crystalline and amorphous. In addition, some curious properties of composites<sup>15</sup> and frictional contacts<sup>16-18</sup> might be clarified.

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## Creeping-film phenomenon of potassium chloride solution

A PECULIAR creeping film that is similar to the superfilm of liquid helium II, but which occurs at room temperatures and has a much lower transfer rate, takes place in saturated potassium chloride aqueous solution. In an electrolytic experiment using saturated KCl solution as the salt bridge, we noticed that without any disturbance the solution could not be kept in the beaker all the time. A thin surface film creeps up the beaker wall very slowly, about several centimetres a day, flows out over the brim and then down the outside surface of the beaker. Creeping can also be observed up the surface of the electrode and the salt bridge tube (Fig. 1). On surfaces over which the film has crept, potassium chloride crystals form as the water in which KCl is dissolved evaporates. Within a few days the solution in the beaker drains out completely and the entire surface of the beaker becomes covered with a thin layer of potassium chloride crystals, about  $10^{-2} \text{ cm}$  thick.

Another anomaly is the leakage of the same liquid solution from a supposedly 'seamless' glass tube connector used for the connection of the salt bridge. The solution flows out through the 'seamless slit', and then spreads all over the outside surface (Fig. 2). The creeping film then evaporates, and crystals form. The creep rate is lower than the previous case. The phenomenon occurs when the connector is



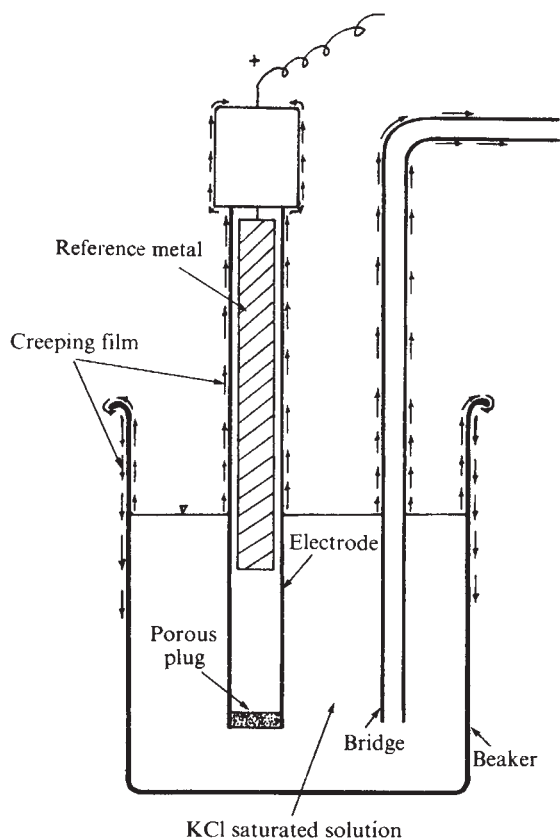
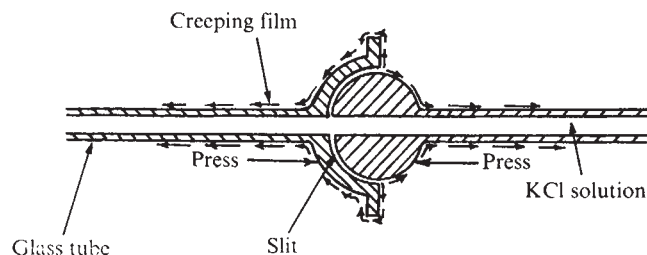


Fig. 1 Surface creep in a set-up including an electrode and a salt bridge.

pressed with a clamp so tightly that water or air, even at very high pressures, cannot pass through.

An experiment was carried out to investigate whether this creeping thin film resulted from the electrical effect of the electrode. Experiments similar to the surface film experiment for liquid helium II were carried out. Instead of the electrode and the salt bridge, two small glass tubes, 1.8 cm in diameter and 11 cm long, were filled with 4 cm and 7 cm, respectively, of saturated KCl aqueous solution and immersed in two 400-cm<sup>3</sup> beakers containing 300 cm<sup>3</sup> of same KCl solution. The glass tubes were immersed in such a way as to provide a difference in level of 3 cm between the liquid in the tube and that in the beaker. To investigate the possible effect of the difference in level, experiment A was performed with the liquid in the tube higher than that in the beaker (Fig. 3). In experiment B the level was lower in the tube. The experiments were carried out with the surface as clean as possible so as to avoid contamination. No observable disturbance was allowed during the experiments. Flow of the creeping film along the surface of the beaker and along the inner and outer surfaces of the tube was observed, with a transfer rate of several centimetres a day.

Fig. 2 Flow through a 'seamless slit' connector.



The film thickness was estimated to be  $10^{-2}$  cm from observation of crystal thickness.

The creeping rate of the liquid inside the tube in experiment A was, however, higher than that observed in experiment B. About two weeks later, the flow spread all over the inner and outer surfaces of the beaker, and started to extend across the bench until the solution had completely drained out. The solution also ascended the tube surface and crept along the supporting string. It is interesting that dried crystals accumulated at the entrance to the tube.

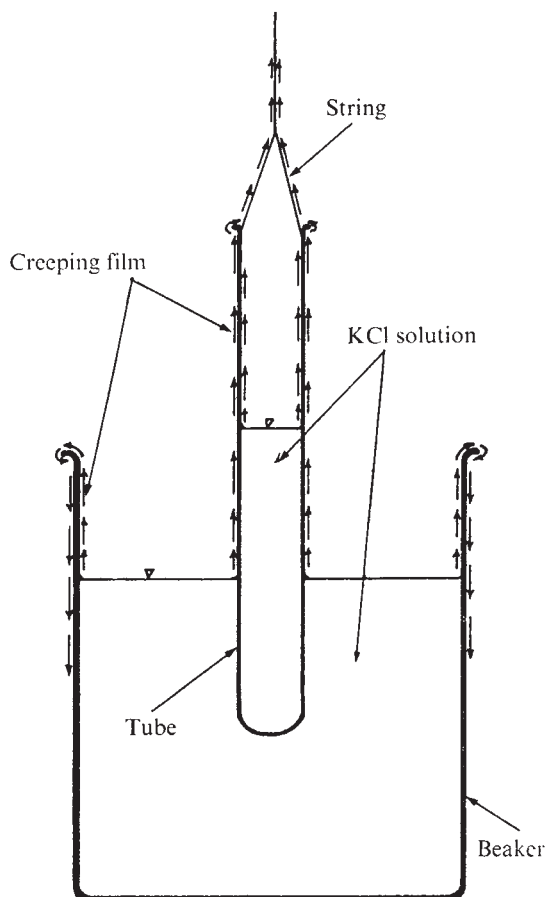


Fig. 3 Experimental set-up without electrode.

After all of the solution in the beaker had drained out, we observed that the heavily accumulated crystal formed a helical shape on the outer wall of the tube and the inner wall of the beaker.

The temperature was maintained at about 22 °C, though slight fluctuations were inevitable. Humidity was not controlled during the experiments. It seems that humidity as well as surface conditions were responsible for the creeping rate of the film. We repeated the experiments using a 'wet' surface, and the transfer rate increased by a factor of about three. But we could not reproduce the experiment from run to run even by using very clean surfaces. It seems that the creep is unlikely to occur if the solution is not saturated (this can happen if the temperature rises so that the solution becomes unsaturated).

This surface film phenomenon cannot be explained in terms of the evaporation of the solution from the bulk liquid level with subsequent condensation on to the surface, because the film creeps over the brim of the beaker. The experiments showed that the phenomenon is not attributable to any electrical effect although that may play a rôle if an electrode is present. No satisfactory interpretations based on present knowledge of either surface chemistry or transport theory can be given. The superfilm phenomenon of

We attribute this phenomenon to the interactions of the ions with the surface of the beaker or tube and the surrounding gaseous molecules; the major driving force is not clear. The leakage through a tightened glass connector is also inexplicable.

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The faunal lists are as shown in Table 1.

Bou Hanifia II

*Hipparion primigenium* (Von Meyer)      *Zramys* cf. *dubius* Jaeger  
*Dicerorhinus primaevus* Aramb.      *Progonomys cathalai* Schaub  
*Orycteropus mauritanicus* Aramb.  
*Palaeotragus germani* Aramb.  
*Samotherium* sp.  
*Damalavus boroccoi* Aramb.  
*Gazella praegaudryi* Aramb.  
*Percrocuta algeriensis* (Aramb.)  
*Propotamochoerus devauxi* Aramb.  
Probosc. indet. (*Deinotherium*?)  
*Progonomys cathalai* Schaub  
*Myocricetodon* sp.  
*Testudo*?  
*Struthio* sp.

# Radiometric age of early *Hipparion* fauna in North-west Africa

A TUFF for which a  $^{40}\text{Ar}/^{39}\text{Ar}$  date of  $12.18 \pm 1.03$  Myr has been obtained is located in the lower level of the Bou Hanifia continental formation (= Oued el Hammam), several metres below the famous *Hipparion primigenium* locality<sup>1-3</sup>. The tuff forms a continuous layer in the lower part of the continental formation and can be observed on either side of the Oued el Hammam Dam. The *Hipparion* fossiliferous horizon (Bou Hanifia I) lies in a second deposit of greatly weathered tuffs and has yielded a few micromammal remains. About 15 m

The Bou Hanifia Formation, about 400 m thick, lies unconformably on a lower Miocene (Cartennian) formation<sup>1,2</sup>, and is overlain by a marine formation assigned to the N17 Zone of Blow<sup>5</sup>. The transition from continental to marine horizons can be observed 6 km to the south of the dam along the N7 road from Sfisef to Ain Fekan, beyond the 'three rivers' bridge. A third micromammal deposit (Sidi Salem) has been discovered there directly below some intermediate brackish deposits with *Potamides* and oysters<sup>4</sup>. Its fauna closely resembles that of the Oued Zra which is 9.7 Myr old, but it contains more highly evolved species. The Sidi Salem deposit is more recent than 9.7 Myr, and is most probably between 9 and 8 Myr old. Thus, the Bou Hanifia Formation covers a time span of around 12–9.7 Myr BP.

**Table 2** Relationships of Middle and Upper Miocene micromammal faunas from northern Africa to marine chronology, radiometric dates and continental chronology

| Age<br>(Myr) | Marine<br>chronology | Radiometric<br>dates | Micromammal<br>faunas                         | Continental<br>chronology | Radiometric dates<br>Europe and<br>tropical Africa | East African<br>calibrated continental<br>formations<br>(Baringo Basin) |                      |
|--------------|----------------------|----------------------|-----------------------------------------------|---------------------------|----------------------------------------------------|-------------------------------------------------------------------------|----------------------|
| 6            | MIOCÈNE SUPÉRIEUR    | MESSINIEN            |                                               | TUROLIEN                  | ← <i>LIBRILLA I</i>                                | LUKEINO FORMATION<br>MPESIDA BEDS                                       | 6                    |
| 7            |                      | N                    | MELKA EL<br>OUIDANE<br>(ex. CAMP<br>BERTEAUX) |                           | KHENDEK EL<br>OUAICH                               |                                                                         | 7                    |
| 8            |                      | 17                   |                                               |                           | AMAMA 2                                            |                                                                         | 8                    |
| 9            |                      |                      |                                               |                           | SIDI SALEM                                         |                                                                         | 9                    |
| 10           |                      |                      |                                               |                           | AMAMA 1                                            |                                                                         |                      |
| 10           | MIOCÈNE MOYEN        | TORTONIEN            |                                               | VALLÉSIEN                 | ← <i>SAMOS 1-4</i>                                 | FEDCBA<br>NGORORA<br>FORMATION                                          | 10                   |
| 11           |                      | N                    | OUED ZRA                                      |                           | OUED ZRA                                           |                                                                         | 11                   |
| 12           |                      | 16                   |                                               |                           | JEBEL SEMMENE                                      |                                                                         | 12                   |
| 13           |                      | N                    |                                               |                           | BOU HANIFIA II                                     |                                                                         |                      |
| 14           |                      | 15                   |                                               |                           | BOU HANIFIA I                                      |                                                                         |                      |
| 12           | MIOCÈNE MOYEN        | SERRAVALLIEN         |                                               | MELLALIEN                 | ← <i>HÖWENEGG</i>                                  | MURUYUR BEDS<br>ALENGERR BEDS                                           | 12                   |
| 13           |                      | 14                   |                                               |                           | PATANIAK 6                                         |                                                                         | 13                   |
| 14           |                      | 13                   |                                               |                           | TESTOUR                                            |                                                                         |                      |
| 15           |                      | 12                   |                                               |                           | BENI MELLAL                                        |                                                                         |                      |
| 16           |                      | N                    |                                               |                           |                                                    |                                                                         | ← <i>FORT TERNAN</i> |
| 17           |                      |                      |                                               |                           |                                                    |                                                                         |                      |
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The period covered by the formation, its relationships with the marine formation, its mammal fauna and the radiometric dates obtained, enhance its importance in the study of the Middle and Upper Miocene of Africa.

Several conclusions can be drawn from the date obtained for the Bou Hanifia tuff.

- The immigration of *Hipparion primigenium* is contemporary in northern Africa and western Europe<sup>6</sup>. The oldest *Hipparion* (*H. primigenium*) known in tropical Africa<sup>7</sup> is slightly more recent than those of northern Africa and Europe. (The meaning of this heterochronism is not yet understood.)

- The immigration of the primitive Murid, *Progonomys cathalai* Schaub, is synchronous with that of *H. primigenium* in the Mediterranean Basin; this has been postulated already<sup>8</sup>, but no radiometric date has been obtained before for a fauna including an association of the two species.

- The pre-Vallesian age of the Beni-Mellal fauna is confirmed<sup>4</sup> establishing that it is more or less contemporaneous with that of Fort Ternan in Kenya<sup>9</sup>. On that basis the association observed in Tunisia of Upper Langhian foraminifers<sup>10</sup> with micromammals similar to those of Beni-Mellal<sup>11</sup> seems explainable<sup>10</sup>.

- The dates complete the biochronological sequence calibrated with rodents of the micromammal localities of the Middle and Upper Maghrebian Miocene (ref. 4, and Table 2).

Table 2 requires further comment:

- *Hipparion primigenium* persists in northern Africa through the Turolian (Melg el Ouidane Deposit). The species is known from around 4 Myr BP in eastern Africa<sup>12</sup>.

- *Hipparion sitifense* Pomel from the Sétif<sup>13</sup> and Mascara<sup>14</sup> lacustrine limestones has been found from the Upper Miocene of Algeria (Amama 2 deposit) and Kenya<sup>16</sup>. So an Upper Pliocene age for the Mascara and Sétif limestones cannot be considered as definitively established.

- An initial conventional K/Ar date of 7.84 Myr was first obtained for the Bou Hanifia tuff. This date contradicted the age indicated by the presence of the Bou Hanifia rodents which are less evolved than those of the Oued Zra deposit ( $9.7 \pm 0.5$  Myr) and did not tie in with the more primitive character of the Bou Hanifia large mammals comparable to those of the oldest Samos localities (Samos 1–4), for which a date of 9.3 Myr years has been obtained<sup>17</sup>. Here again, the evolution of rodents provides a particularly dependable tool for the elaboration of continental biostratigraphic scales. The interpretation of the full <sup>40</sup>Ar/<sup>39</sup>Ar age spectrum analysis of biotite from the Bou Hanifia tuff is that it was altered during early cementation/diagenesis around  $12.18 \pm 1.03$  Myr BP; the age of sedimentation must have been a little earlier.

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## Orientation specific colour adaptation at a binocular site

McCOLLOUGH<sup>1</sup> found that after alternate viewing of vertically oriented red gratings and horizontally oriented blue gratings, achromatic gratings appear tinted blue when oriented vertically and pink when oriented horizontally. This indicates the existence of colour specific edge detectors in the visual system. Further, since interocular transfer of the effect was not possible, McCollough concluded that no binocular cells are involved in this type of adaptation. This view has been supported by many investigators<sup>2–5</sup> who used different paradigms. But their failure to illustrate orientation-contingent colour adaptation at a binocular locus may be attributable to the fact that most of their experimental situations, like McCollough's, did not provide the ideal stimulus for binocular neurones, which respond best, showing "binocular synergy", only when the stimulus is simultaneously presented to both eyes<sup>6–9</sup>. I have attempted optimal excitation of the binocular cells by alternately presenting red vertical and blue horizontal gratings to both eyes and isolating adaptation of binocular neurones by cancelling monocular adaptation with presentation of a pair of complementary gratings (blue vertical and red horizontal) to each eye separately for an equal length of time. The results of this procedure show that adaptation does involve binocular neurones.

If only the monocular channels are colour coded, no aftereffect should be obtainable on viewing an achromatic test pattern, since the group of monocular neurones specific to any of the two orientations have been presented with both the complementary colours for equal periods of time. On the other hand, if any of the binocular orientation detectors are colour contingent, an aftereffect complementary to the binocular presentation, namely, an achromatic test grating appearing bluish when vertical and pinkish when horizontal, should be seen on viewing the test stimulus with both eyes. I am aware of MacKay and MacKay's argument<sup>10</sup> against selective adaptation of single units sensitive to colour and orientation, but since it is not contradictory to my central theme I shall talk of "selective adaptation of cells", and leave discussion of their hypothesis for the final interpretation.

Coloured rectangular gratings (dark : light = 1 : 2) of 2.5 cycles per degree, subtending a visual angle of 15° at a distance of 8 feet were presented to five naive observers with normal form and colour vision in the following sequence (colour filters used were Wratten Nos 25 and 44): red vertical, binocular; blue vertical, right eye; red horizontal, right eye; blue horizontal, binocular; red horizontal, left eye; blue vertical, left eye. Each of the above presentations was for 8.5 s with a dark interval of 1.5 s between successive presentations, and the whole cycle was repeated for a total duration of 30 min. When these observers were later shown a test pattern containing achromatic gratings of both vertical and horizontal orientations, all of them

reported seeing bluish vertical and pinkish horizontal gratings when both the eyes were open; with either eye occluded they all reported seeing pinkish vertical and bluish horizontal gratings. No leading questions were put to them and their choice of colour tints on viewing the test stimulus was always spontaneous. The aftereffects could be easily obtained up to 24 h after the adaptation, comparable to the time-course of the classical McCollough phenomenon.

These observations point to the colour specificity of two types of binocular cells: (1) cells which respond much more strongly for a binocular stimulus than for a monocular one. Electrophysiologically, most binocular units have been shown to be of this type<sup>6-9</sup>, though their colour specificity has not been clearly demonstrated. (2) Neurones which receive a facilitatory input from one eye and an inhibitory signal from the other. The simple cells in area 17 reported by Hubel and Wiesel to be monocularly driven<sup>6,11</sup> have been shown<sup>12</sup> to receive a binocular input in the cat. Though these cells are excited only through the "dominant" eye, an "incorrectly" placed stimulus in the non-dominant eye has a strong inhibitory effect. A typical receptive field in the non-dominant eye has a large inhibitory surround and a "non-inhibitory" centre. The gratings that we used in our experiment, when binocularly presented, are bound to excite the inhibitory surround in the non-dominant eye's receptive field, and consequently suppress the response of the cortical cell, even though the dominant eye may be receiving the right stimulus. Thus these units would respond to the lines of a grating, when they are presented to the dominant eye alone, but not for a binocular presentation. If neurones such as these could be involved in a colour-specific adaptation, it would explain our observers reporting pinkish vertical and bluish horizontal aftereffects when one of the two eyes was occluded.

MacKay and MacKay, after measuring the time-course of the McCollough effect, questioned<sup>10</sup> the concept<sup>1</sup> of selective adaptation of single units sensitive to colour and orientation, and suggested, alternatively, cooperative synaptic and subsynaptic changes among an associative network of neurones. I suggest that these changes could occur at a binocular site too. The binocular units may not themselves be colour coded, but they are at least actively involved in the hypothetical neuronal changes. The claims that the McCollough effect is confined to monocular channels<sup>1-5</sup> or that it could be just retinal<sup>10</sup> are not tenable. It can even be proposed that all McCollough adaptation occurs in the visual cortex, in accord with the electrophysiological findings of cells with only concentric receptive fields before the striate cortex in the visual pathway<sup>13</sup>.

My results are consistent with the extensive neurophysiological evidence for colour coded cells in the visual cortex<sup>14-19</sup>. Though only a few of these cells possess both colour and spatial specificities<sup>16</sup>, it can be suggested that all the colour sensitive neurones, whether they have orientation specificity or not, are involved in structural changes with orientation-specific binocular neurones to yield long lasting aftereffects contingent on colour and orientation. This model is basically similar to the one proposed by Leppmann<sup>20</sup>. The relative sparsity of cells with both specificities and the hypothetical neural changes justifies the requirement of a strong cellular response (binocular synergy) to bring out an aftereffect, in contrast to the easy interocular transfer<sup>2</sup> of pattern adaptation.

My observations are also the first psychophysical correlates of some of the stimulus properties of the various binocular neurones described with electrophysiological techniques, namely, the facilitated binocular response<sup>6-9</sup> and the subtle inhibitory input from an otherwise uninfluential eye<sup>12</sup>. It would be interesting to apply our experimental paradigm to other phenomena, such as aftereffects contingent on colour and movement, that do not show<sup>21</sup> interocular transfer.

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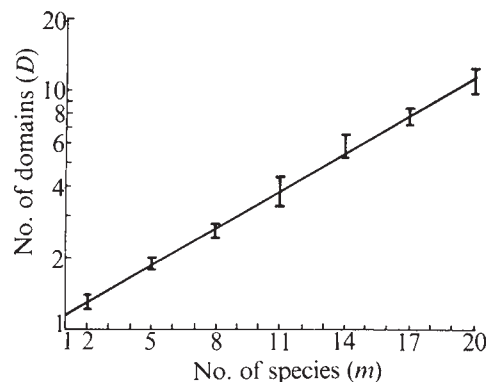
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## Multiple domains of attraction in competition communities

ECOLOGISTS typically explain the absence of a species in an area by searching for ultimate climatic or physical factors that limit its distribution or favour its competitors. The possibility that the system is not in equilibrium—that colonisation has not occurred—is not normally considered. Even less appreciated is that there may be a number of alternative stable equilibrium communities which may develop in a given area and that the final outcome may depend solely or partially on historical factors such as the sequence and numbers in which each species colonises. Here we investigate the theoretical possibility of this proposition for competition communities.

Examples of multiple domains of attraction are present in intertidal communities<sup>1</sup>, microbial systems<sup>2</sup> between flour beetles<sup>3</sup> and on defaunated mangrove islands<sup>4</sup>. Diamond's study of competition among 400 bird species on the several hundred islands off the New Guinea archipelago offers some excellent examples<sup>5</sup>. He was forced to go beyond the normal competition theory and to introduce the concept of community assembly rules (forbidden and permitted subsets of species) and transition probabilities between them. We here investigate some of the dynamical underpinnings of species assembly rules.

**Fig. 1** Number of domains of attraction against the initial number of species in the competition system. The mean interspecies competition ( $\bar{a}$ ) is 1.0. Bars indicate  $\pm 1$  s.e.





Unfortunately, it is impossible to analyse analytically such global stability problems as this for large numbers of interacting species. The local (or Liapunov) stability analyses of ecological communities of May<sup>6</sup>, Gilpin<sup>7</sup>, Roughgarden<sup>8</sup> and others is inappropriate here. We have opted to explore state-space landscape of interspecific competition communities by numerically integrating a normalised version of the Lotka<sup>9</sup> and Volterra<sup>10</sup> equations:

$$\frac{dN_i}{dt} = N_i(1 - a_{ij}N_j) \quad (1)$$

$$i = 1 - m, \quad a_{ii} = 1, \quad a_{ij} \geq 0$$

where  $N_i$  is the population density of the  $i$ th species and  $a_{ij}$  is the competition of the  $j$ th species on the  $i$ th species. Each species has a low density exponential growth rate of 1 in the absence of other species and reaches a single species equilibrium of 1. For a random assignment of the  $a_{ij}$  we wish to know the number of domains of attraction of the system and their probability of occurrence. We could do this by testing the feasibility, stability and resistance to invasion of the  $2^m$  different combinations of the  $m$  species. But this is very costly for large  $m$ , and, moreover, tells nothing about the probability of occurrence of the different domains of attraction. We have therefore chosen to randomly initialise the system on the hyperplane  $\sum N_i = 1$ . Thus, each initialisation corresponds to one possible combination of relative abundances of the potential colonising species pool. Equation (1) is then solved by computer iteration until a stable domain is reached. During the course of this iteration certain species will become extinct. It is theoretically possible that a limit cycle (or perhaps ergodic behaviour) would result, but in the several hundred systems we explored we did not find any such case. Perhaps this was due to the biologically realistic truncation used—any species that fell below one one hundred thousandth of its single species equilibrium went extinct.

We initially used colonising pools species ( $m$ ) of size 2, 5, 8, 11, and 14. Values of  $a$  were drawn from a rectangular distribution with a mean interspecies competition coefficient ( $\bar{a}$ ) of 0.35, 0.5, 0.65, 0.8 and 1.0. In one series the  $a$  matrix was symmetrical; in another all the terms of the  $a$  matrix were random; in a third series the  $a$  matrix was half a symmetrical matrix plus half a random matrix. For each  $m$  and  $\bar{a}$  combination, 10 matrices were generated each of which was solved with 20 different initialisations. A multivariate analysis of the resulting data—that is, the number of domains of attraction compared with community size and mean strength of interspecies competition—produced a single parameter model that explained almost 90% of the experimental variance

$$D = \exp[p(m-1)\bar{a}] \quad (2)$$

where  $D$  is the number of domains of attraction and  $p$  is an empirical parameter. For the symmetrical, mixed and random matrices, respectively,  $p$  has the values ( $\pm$ s.e.)  $0.182 \pm 0.025$ ,  $0.084 \pm 0.014$ , and  $0.062 \pm 0.011$ . Thus competition matrices which are symmetric, as might be the case for resource competition between species with equal niche breadths and single species equilibria, give more domains of attraction than non-symmetric competition. The latter might occur where there is interference competition or where there is much dimensionality in niche packing.

To test empirical relation (2) for the functional relationship between  $D$  and  $m$ , we ran a more exhaustive series of tests in which each competition matrix was initialised 200 times; the  $a$  matrices were half symmetrical and half random and  $\bar{a}$  was 1.0;  $m$  was varied between 2 and 20 in steps of 3. The results are shown in Fig. 1. The logarithmic relationship between  $D$  and  $m$  is confirmed, although the regression line misses the point ( $D = 1, m = 1$ ), passage through which is logically necessary, by a few per cent. This could be corrected by adding a small constant to the right hand side of equation (2).

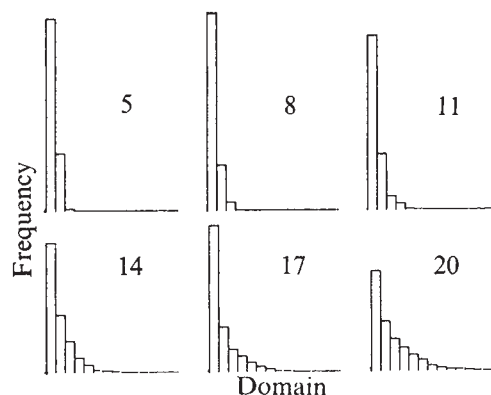


Fig. 2 Relative frequency of the domains of attraction for different initial community sizes. The domains are ordered by decreasing the frequency.

It is clear that relation (2) cannot hold for arbitrarily large values of  $\bar{a}$ , since for any  $m$  there exists an upper bound on the possible number of alternative equilibria. In the range of  $\bar{a}$  that we investigated, however, the exponential dependence of  $D$  on  $\bar{a}$  was a better fit than a linear dependence.

The relative 'volumes' of the domains of attraction were determined by counting the number of times a particular competitive assemblage occurred over the 200 times each  $a$  matrix was initialised. In Fig. 2 these are plotted with histograms. The bar on the far left represents the average frequency of the most common domain for the 10 different competition matrices, the next bar is the next most frequent domain and so on. As  $m$  increases the distribution spreads out but the relative volume of the largest domain of attraction decreases only slightly.

In Fig. 3 we have plotted both the mean number of species in a domain (weighted average over all domains,  $a$  matrices, and initialisations) and the mean total density of species in the domains as a function of the number of possible colonising species ( $m$ ), for an average  $\bar{a}$  of 1.0. These asymptote at ordinate of 2.5 species and 1.4 times the single-species equilibrium density, respectively, at an  $m$  of about 10.

A particular solution with a small domain of attraction may be easily perturbed by species increases or random perturbations to a solution with a broad basin of attraction, from which only larger perturbations will knock the system into other potential domains. It is interesting in this context that there is no regularity with the number of species present in a particular solution and the size of the domain of that solution for a particular  $a$  matrix. For example, stable domains containing both very few (for example, one) and large number of species, may be relatively unstable to global perturbations. Neither have we been able to discern any obvious heuristic rules in our trials which enable us to predict the attributes of the largest domains of attraction based on either species number,

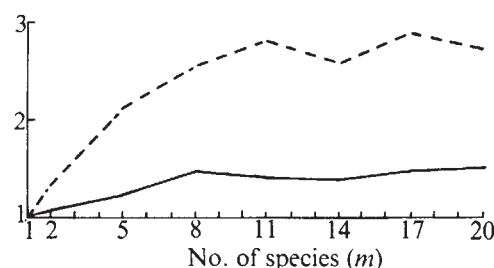


Fig. 3 Mean number of species present in a domain of attraction against initial number of species in the community (dashed line) and the mean total density  $\sum N_i$  of the species in a domain of attraction against the initial number of species in the community (solid line).



total density of all species, or combinations of these variables.

Since these results come from equations that are no more than a caricature of ecological reality, our results must be taken as provisional. Nonetheless, some interesting and practical conclusions may be drawn. Pest eradication programmes or the introduction of biological control agents may be viewed in some situations as global perturbations of community equilibria, or as attempts to go from one stable domain of attraction to another which is economically or aesthetically more desirable. The likelihood of success will depend on the relative size of the domain of the existing community compared with the size of the desired community, and the size and direction of the manipulated perturbation (for example, the number of individuals introduced). In some situations there may only be a few starting configurations of colonisation sequences leading to the formation of an economically desirable community. The lack of initial success in a species introduction plan does not necessarily mean that success will not be reached using another stocking rate or at a different time when resident species numbers are different.

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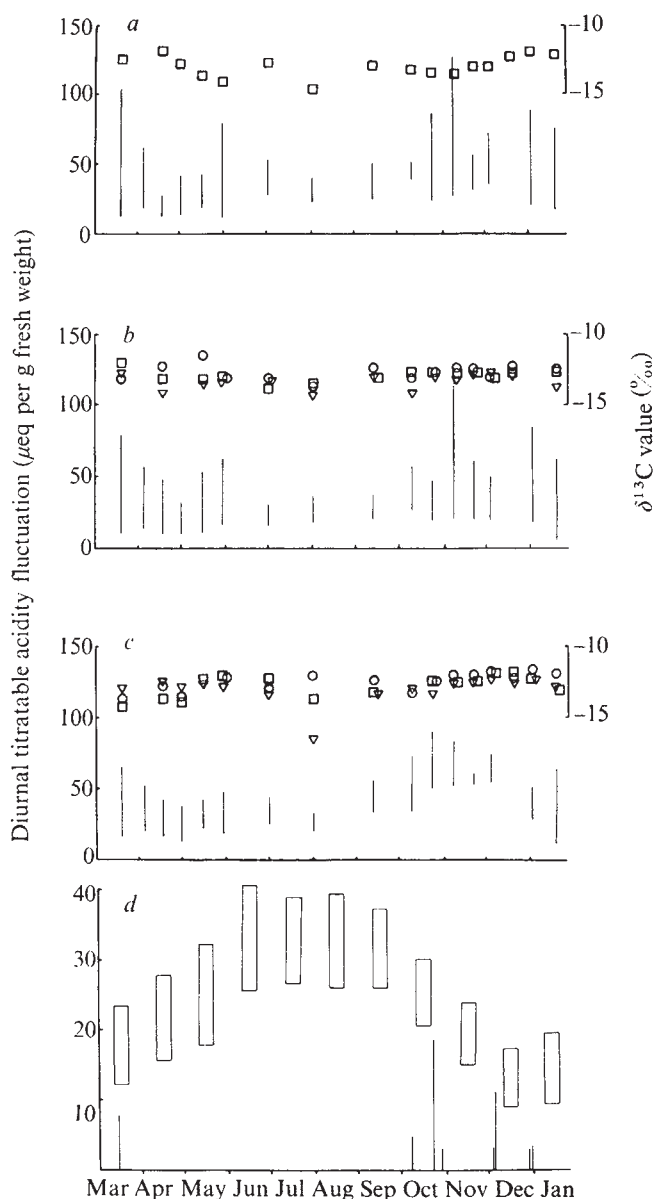
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## Seasonal effects on carbon isotope composition of cactus in a desert environment

In plants with crassulacean acid metabolism (CAM),  $\text{CO}_2$  is fixed in the light by ribulose-1,5-bisphosphate carboxylase<sup>1,2</sup> and in the dark by phosphoenol pyruvate (PEP) carboxylase<sup>2,3</sup>. Because these two enzymes fractionate the stable isotopes of carbon ( $^{12}\text{C}$  and  $^{13}\text{C}$ ) in the atmosphere and discriminate against  $^{13}\text{C}$  to different extents<sup>4</sup>, the wide range of values of carbon isotope discrimination ratios ( $\delta^{13}\text{C}$ ) found in these plants<sup>5</sup> has been attributed to different contributions of dark and light fixation to carbon gain<sup>6</sup>. The proportion of total carbon fixed at night can be altered by environmental variables such as temperature<sup>7</sup>, water status<sup>8</sup> and photoperiod<sup>3</sup>. Predictable changes in  $\delta^{13}\text{C}$  values have been observed when these environmental conditions have been varied. With one exception, all such studies have been performed in artificial conditions. Because the  $\delta^{13}\text{C}$  ratio of CAM plants has been used as an indicator of environment in palaeoecological investigations<sup>9</sup>, we have examined the sensitivity of these  $\delta^{13}\text{C}$  ratios to seasonal environmental influences in a natural system. Using *Opuntia acanthocarpa*, *O. basilaris* and *O. bigelovii*, we found that the carbon isotope composition of the plants did not change during the experiment, indicating the insensitivity of this measurement to short term seasonal influences in this environment.

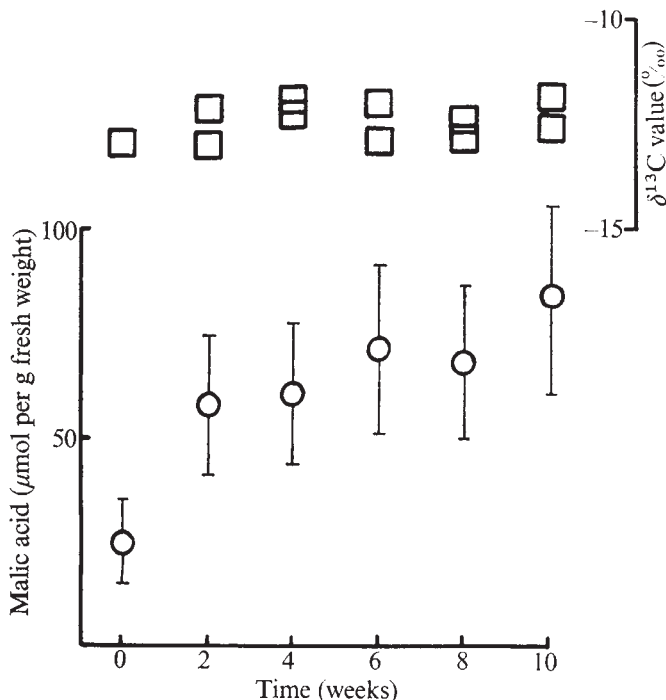
The experimental site was at an altitude of 289 m in

the Phillip L. Boyd Desert Research Centre at Palm Desert, California, and had a climate characterised by the temperature and rainfall pattern shown in Fig. 1. Tissue for each analysis was sampled by taking cork-borer cores transversely through the cactus cladodes. Each determination<sup>10</sup> was based on at least three cores and for *O. basilaris* and *O. bigelovii*, they were sectioned into cortex and pith, using the vascular strands as a guide for the division. Total titratable acidity and malic acid content<sup>3</sup> were determined in addition to the  $\delta^{13}\text{C}$  ratio. Figure 1 shows that the extent of the diurnal change in titratable acidity responded to rainfall, particularly in the autumn. These results agree with those obtained previously with *O. basilaris* on the same site<sup>11,12</sup>. In the previous study, nocturnal stomatal opening occurred briefly after rain, facilitating dark  $\text{CO}_2$



**Fig. 1** Diurnal titratable acidity fluctuations (bars) and  $\delta^{13}\text{C}$  values in natural populations of *O. acanthocarpa* (a) *O. basilaris* (b) and *O. bigelovii* (c) sampled in an environment with (d) the indicated mean monthly temperature ranges (blocks) and rainfall (bars). □, Bulk tissues; ▽, cortex; ○, pith. Titratable acidity was determined by titration of boiled aqueous tissue extracts to pH 7.0 with 0.01 N KOH. Correlation coefficients between  $\delta^{13}\text{C}$  ratios and diurnal acidity changes: a, -0.0503; b, -0.3155; c, 0.1308; and time: a, -0.035; b, -0.209; c, -0.290. Mean annual  $\delta^{13}\text{C}$  value and s.e.m.: a,  $-13.1 \pm 0.193$ ; b,  $-13.0 \pm 0.085$ ; c,  $-12.8 \pm 0.121$ .

fixation which resulted in increased diurnal fluctuations in acidity. At other times, stomata remained closed throughout the day and the small diurnal changes in acidity observed in these conditions were assumed to correspond to nightly fixation of respiratory  $\text{CO}_2$  only. In no conditions did stomata open in the light. This pattern of  $\text{CO}_2$  uptake would involve only PEP carboxylase in the assimilation of atmospheric  $\text{CO}_2$ , and from this a stable  $\delta^{13}\text{C}$  ratio of the magnitude observed in  $\text{C}_4$  species would be predicted. Such a ratio was observed in this experiment (Fig. 1). Statistical analysis of the results indicated that the  $\delta^{13}\text{C}$  ratios of *O. acanthocarpa* and *O. bigelovii* were correlated with neither the time of year nor the diurnal change in titratable acidity. The correlation between  $\delta^{13}\text{C}$  value and diurnal fluctuation in titratable acidity for *O. basilaris* was significant ( $P=0.05$ ). But, the range of  $\delta^{13}\text{C}$  values observed for all species was within the limits of observation and  $\text{C}_4$  species<sup>3</sup>. In *O. basilaris* and *O. bigelovii* the  $\delta^{13}\text{C}$  ratios measured in pith and cortex samples were not significantly different from the bulk value for the tissue. In other experiments (our unpublished data), although carbon fixed at night was initially found localised in the cortex, radial equilibration with the pith occurred in a few days.



**Fig. 2** Response of morning *O. bigelovii* malic acid levels and  $\delta^{13}\text{C}$  values to irrigation in autumn. A natural clone of plants was irrigated with 6–10 mm of water every 2 weeks. Correlation coefficient between  $\delta^{13}\text{C}$  values and time was 0.412. Mean and standard error of the  $\delta^{13}\text{C}$  values was  $-12.6 \pm 0.137$ . Vertical bars indicate 95% confidence limits for the malic acid estimation.

The sensitivity of the  $\delta^{13}\text{C}$  ratio to environmental change was further examined in a clone of *O. bigelovii* which was subject to regular irrigation in autumn, when the cooler days and improved water status of the plants was expected to promote daytime  $\text{CO}_2$  fixation<sup>8</sup>, and therefore a decline in the  $\delta^{13}\text{C}$  ratio. The results, presented in Fig. 2, indicate that although the production of malic acid at night increased markedly, the  $\delta^{13}\text{C}$  ratio remained stable at a high value. The data presented here therefore indicate that the  $\delta^{13}\text{C}$  ratio of the plants studied is relatively insensitive to short term seasonal changes in natural desert conditions. The data also confirm that no exogenous  $\text{CO}_2$  is fixed in the light in this environment. These experiments imply

then, that the varying  $\delta^{13}\text{C}$  ratios observed in wild populations of cactus<sup>1,9</sup> are a result of long term environmental differences rather than seasonal effects. This result gives credence to the use of such ratios for the reconstruction of past climates<sup>9</sup>.

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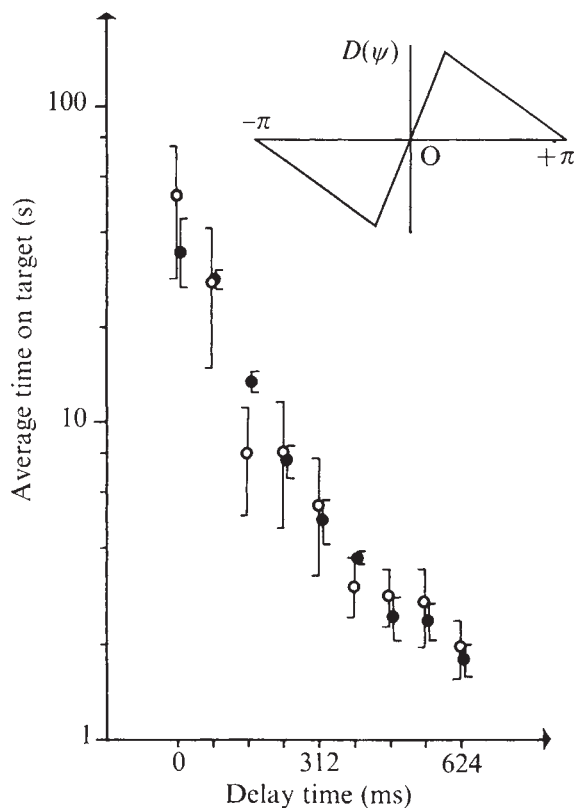
## Real-time delayed tracking in flies

A DARK target on a bright background is fixated<sup>1</sup> and tracked<sup>2,3</sup> by a flying fly. The delay (dead time) in the fly's behavioural response to a change in the retinal image of the target is very small (about 20 ms)<sup>3–5</sup>. An artificial increase of this delay may give useful insight into the kind of control systems used by the tracking fly. Comparable psychophysical tracking experiments have been performed. For instance, when a televised display of a person's own behaviour in (closed-loop) pursuit tracking is delayed, his performance, as measured by time on target, is seriously impaired<sup>6</sup>. It is possible to delay the fly's response in artificial closed-loop conditions, and we have therefore used the effects of delayed visual feedback on tracking performance to test quantitatively a theoretical description of the visual orientation behaviour of flies<sup>1,7,8</sup>.

A female fly (head fixed to the thorax) was attached to a fast torque meter, in the centre of a cylindrical panorama, as described before<sup>1</sup>. The torque signal is transduced into angular displacement of the 'panorama' through an analog simulation of the flight dynamics. In this way it is possible to simulate a free flight situation, in so far as the fly can track a target moving in the horizontal plane. In our experiments the moving target consisted of a black vertical stripe. The angular speed of the target was determined by a zero-mean Gaussian noise added to the torque signal of the fly. The torque,  $F$ , of the fly was delayed in steps of 78 ms by means of a standard tape recorder. The experimental situation is described by the following equation

$$[\theta\ddot{\psi}(t) + k\dot{\psi}(t)] + k\omega(t) = -F(t - \epsilon) \quad (1)$$

where the angular error  $\psi(t)$ , represents the actual position of the target on the fly's retina. The terms in brackets on the left give the analog approximation of the flight dynamics ( $\theta$  is the moment of inertia of the fly,  $k$  represents a rotational



**Fig. 1** Tracking performance under delayed response. The data points represent the mean time on target for six female flies, *Musca domestica* L. (○) and for four analog simulations of equation (1) (●). The vertical bars indicate the standard deviation of the mean. The inset shows the response function  $D(\psi)$  which gives the  $\psi$ -dependent 'attractiveness' of a black stripe for the fly.  $D(\psi)$  and the other parameter values used in the simulation are the same as those measured by Reichardt<sup>1</sup>. With these values, equation (2) without the terms  $N(t)$  and  $k\omega(t)$  shows oscillatory solutions for  $\epsilon \geq 156$  ms. Correspondingly, the associated linearised equation becomes unstable.  $\omega(t)$ , the target angular velocity, is a Gaussian zero-mean process in all experiments, with a flat spectrum up to 15 Hz and an r.m.s. amplitude of about  $50^\circ \text{ s}^{-1}$ . The width of the stripe was  $2^\circ$ , and its vertical length about  $60^\circ$ .

friction constant).  $\omega(t)$  is the zero-mean Gaussian process representing the angular speed imposed on the target in our experiment. The average time on target  $T$  was measured as a function of the artificial delay  $\epsilon$ . The object was considered to be lost by the fly when the angular error  $\psi(t)$  between the direction of flight of the fly and the object reached  $\pm 170^\circ$ .

In tracking behaviour, an increasing delay impairs performance: the greater the delay, the smaller is  $T$  (Fig. 1). The relationship between delay and time on target does not seem to fit a simple function. Psychophysical experiments, on the other hand, seem to show a simple inverse linear relationship between  $T$  (on a logarithmic scale) and magnitude of delay<sup>8</sup>. The orientation behaviour of flies and various tracking situations can be described quantitatively by a phenomenological theory<sup>2-4,7-14</sup>, which leads to the equation

$$\theta\ddot{\psi}(t) + k\dot{\psi}(t) + k\omega(t) = -D[\psi(t)] - r\dot{\psi}(t) + N(t) \quad (2)$$

where the right hand side describes the instantaneous torque of the fly.  $N(t)$  is a zero-mean Gaussian, band-limited random process, independent of visual input;  $r\dot{\psi}$  represents a velocity-dependent, direction-sensitive optomotor response;  $D(\psi)$  is a nonlinear anti-symmetrical function describing the fly's response to the stripe position (inset Fig. 1 and legend). All these terms have been characterised quantitatively<sup>1,3,4,7</sup>.

If the right hand side is delayed by  $\epsilon$ , equation (2) should represent our experimental situation. An approximate analytical

solution in  $T$  has been obtained<sup>9</sup> in the case  $\epsilon = 0$  but seems otherwise extremely difficult. Therefore, to check the validity of the model, we carried out an analog simulation of equation (2), using the parameter values measured by Reichardt<sup>1</sup>.  $T$  was measured in the same conditions and with the same experimental arrangement as was used for the fly (Fig. 1). In the simulation we neglected the internal delay time of the fly (20 ms), and our results are consistent with previous evidence<sup>3,7</sup> that it can indeed be neglected.

The satisfactory agreement between the two sets of data of Fig. 1 supports the validity of the hypothesis underlying the phenomenological theory. Not only do the quantitative values of  $T$  as a function of  $\epsilon$  agree within the limits of experimental error, but also the autocorrelations of the process  $\psi(t)$  for the various values of  $\epsilon$  are similar. In summary, equation (2), in addition to explaining various orientation situations quantitatively, also accounts for the delayed tracking described here. An important consequence is that the range of validity of the terms  $D(\psi)$  and  $r\dot{\psi}$ , representing the fly's visual induced response, extends through the rather extreme tracking conditions of our experiment. In particular, the function  $D(\psi)$ , which to a great extent determines the tracking performance, seems to be an almost invariant property of the visual control system of the fly.

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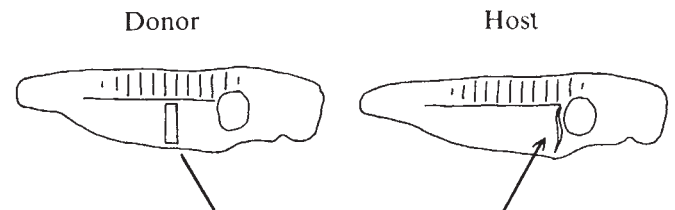
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## Determination of polarity in the amphibian limb

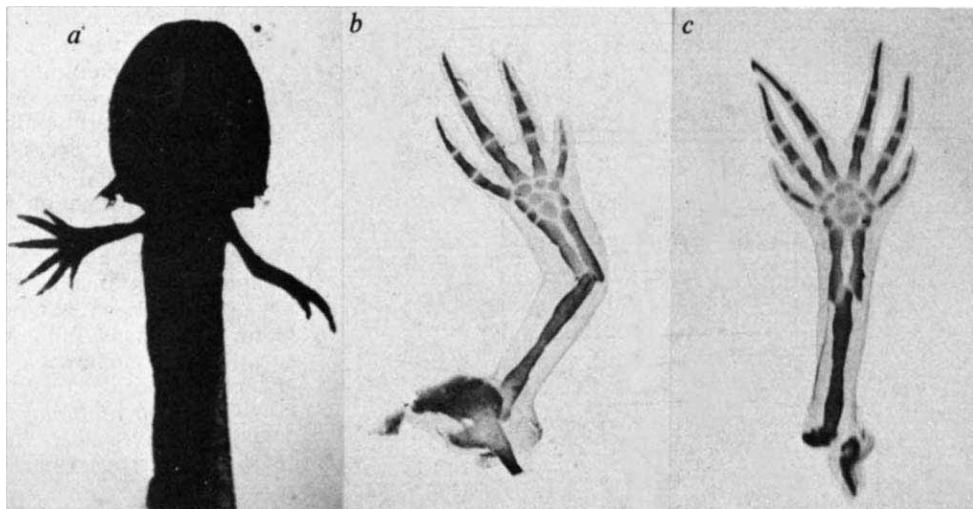
ACCORDING to the classical work of Harrison and his followers<sup>1-6</sup>, the anteroposterior polarity of amphibian limbs consists of a 'molecular polarisation' of the cells which has been acquired by the late gastrula stage of development. I here present evidence that the polarity of the anteroposterior pattern is caused at a later stage of



**Fig. 1** Diagram of the reduplication-inducing graft described in the text. Operations were performed with electrolytically sharpened tungsten needles. The embryos were in half-strength Holtfreter solution containing 1/3,000 MS222 (Sandoz). The grafts were kept in place for 1-2 h with glass bridges. Embryos were kept in half-strength Holtfreter until the wounds had healed (usually overnight) and then transferred to 1/10 × Holtfreter containing 1/10,000 Nystatin (Squibb) and 1/2,000 Sulphadiazine (May and Baker). After hatching, larvae were kept in tap water which had been left to stand for 24 h. They were fed on brine shrimps (California Brine Shrimps Inc.) until all of the limb cartilages had developed, which takes about 4 weeks at  $18^\circ \text{C}$ .



**Fig 2** *a*, A reduplicated limb *in situ* showing its unusual posture; *b*, normal axolotl limb; *c*, reduplicated axolotl limb. For (*b*) and (*c*) the limbs were fixed in 5% trichloroacetic acid for 3 h, bleached overnight in Mayer's bleach, stained for 30 min with 0.1% Alcian green in acid alcohol, differentiated overnight in acid alcohol, dehydrated and cleared in oil of wintergreen.



development by a signal emitted from a region posterior to the limb rudiment. In so far as this signal can travel in both directions it is not necessary to postulate any polarity at the cellular level.

The basic experiment which demonstrates the existence of the polarising region is shown in Fig. 1. It was performed on stage-34 embryos of the axolotl (*Ambystoma mexicanum*)<sup>7</sup>. A strip of tissue consisting of ectoderm and lateral plate mesoderm was grafted from a position ventral to the 6th somite into a slit made ventral to the junction of somites 2 and 3, that is, from the posterior to the anterior margin of the limb rudiment. In 15 out of 25 cases the host forelimb developed into a mirror symmetrical reduplication. These reduplications consist of two posterior halves arranged symmetrically around the mid-axis. Typically, they have five to seven digits and a corresponding number of more proximal elements (Fig. 2*b* and *c*). The pattern of outgrowth of the buds is also symmetrical. The direction of growth is lateral, rather than dorso-posterior, so that the reduplicated limbs stick straight out from the body when they are fully developed (Fig. 2*a*).

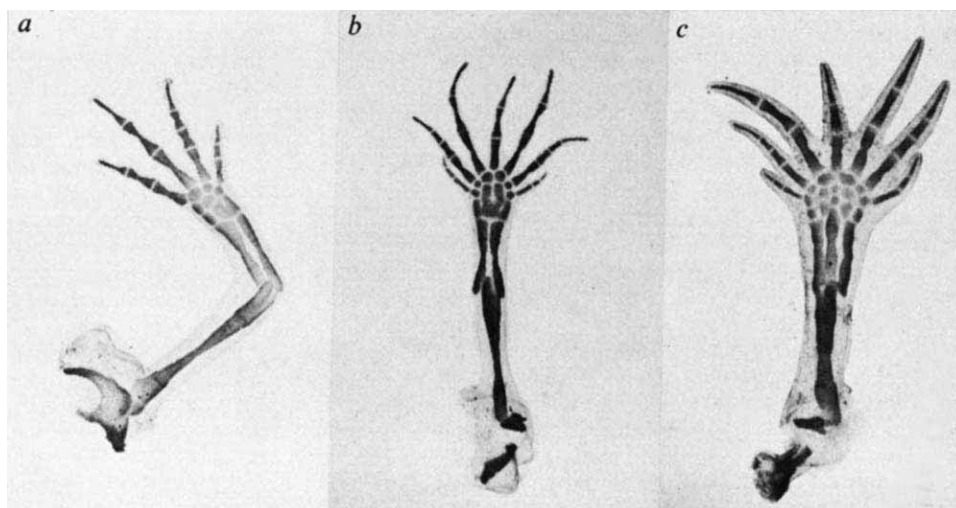
When the grafts were taken from an anterior position (ventral to the 3rd somite), the host limbs developed normally (6 of 6 cases), suggesting that the phenomenon is not a nonspecific wounding or grafting effect. When such anterior strips were grafted posterior to the limb rudiment (ventral to the 6th somite) the host limbs also developed normally (6 of 6 cases). So the cause of the reduplications cannot simply be a disharmonic combination of anterior and posterior tissues.

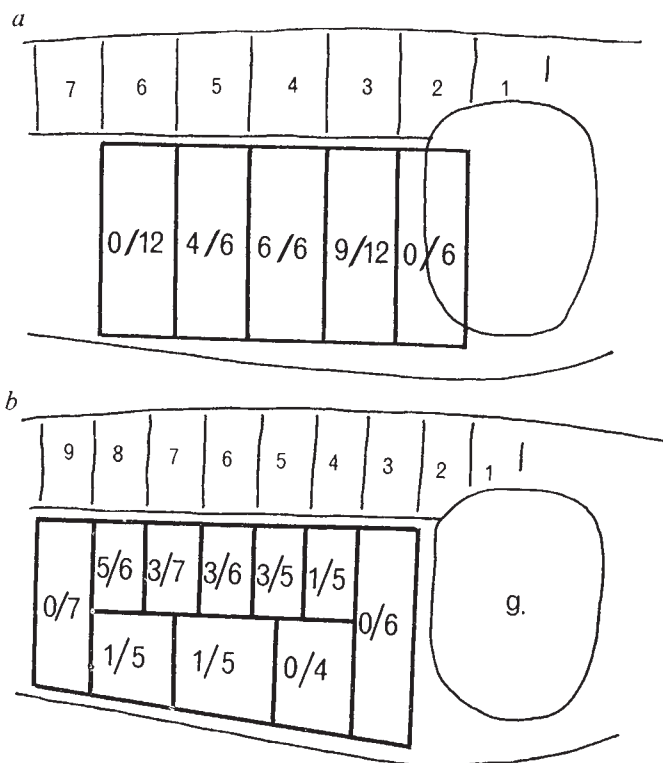
The main reason for thinking that the grafts of posterior origin emit some form of signal is that the reduplications are formed entirely from host tissues. There are several lines of evidence for this, but the most clear cut is that the effect can be produced by an interspecific graft from embryos of the Spanish salamander (*Pleurodeles waltlii*) to the axolotl, and the resulting reduplications consist entirely of axolotl-type elements (Fig. 3). The same may be shown by the use of vitally stained grafts. It therefore seems that the signal from the polarising region is not instructive. What it does in this experiment is to evoke the formation of normal host elements in an abnormal position.

The approximate extents of the polarising and competent tissues were determined by further grafting. Pieces of tissue from the flank were assayed for polarising activity by studying whether or not they produced reduplications when grafted anterior to the limb rudiment. Other strips of tissue were assayed for 'limb competence' by being grafted to the flank between the fore and hind limb rudiments. Because of the extensive regulative ability of this system such fragments can develop into complete supernumerary limbs if they are competent (Fig. 4). It is clear that the zone of limb competence lies ventral to somites 3–5 as described by Harrison<sup>1</sup>. But the polarising region extends well beyond this, the greatest activity lying in the dorsal lateral plate ventral to somites 5–8. Thus, although it is not possible to prove the existence of a sharp boundary by grafting experiments, there is certainly a considerable geographical separation between the two types of tissue.

It is now possible to explain the occurrence of redupli-

**Fig. 3** *a*, Normal limb of *Pleurodeles waltlii*; note the spindle-shaped phalanges and the fusion of intermediale and ulnare in the proximal row of carpals. *b*, Reduplication produced by a pleurodele-pleurodele graft; all cartilage elements are of pleurodele character. *c*, Reduplication produced by a pleurodele-axolotl graft; all cartilage elements are of axolotl character.





**Fig. 4** *a*, Distribution of limb competence in stage-34 axolotl embryos, as assayed by the ability of fragments to form limb buds when grafted to the flank. *b*, Distribution of polarising ability at the same stage, as assayed by the ability of fragments to produce reduplication of the host forelimb when grafted ventral to the junction of somites 2 and 3. *g*, Gill rudiment. For both (*a*) and (*b*) the numbers in each box represent the number of positive cases over the total number of grafts for this particular fragment of tissue.

cations in the earlier, classical experiments. Most of that work involved rotations and transplantations of the so-called 'limb disks'. These consisted of lateral plate tissue extending from the level of the 6th somite to the anterior edge of the 3rd and therefore must have contained both polarising and competent tissue.

When such a limb disk was grafted to the normal site in normal orientation it gave a normal limb. When it was grafted to the normal site in reversed anteroposterior orientation it gave a reduplicated limb<sup>2</sup>. This is presumably because in the former case the polarising tissue remained in its usual posterior position, but in the latter case it was present on both sides of the competent tissue, as in the experiments described above. When a limb disk was grafted to the flank with reversed anteroposterior orientation, it gave a normal limb of reversed polarity. When the graft was normally orientated on the flank it often gave a reduplication<sup>2</sup>. This result was particularly mystifying to Harrison because the reduplications arose from the graft which was 'harmonic' with respect to the body axis. It is no longer surprising, however, because it can now be seen that it was the normally orientated grafts which had polarising tissue on both sides.

In addition, it is probably the polarising region that is responsible for the polarity of induced limbs. Balinsky<sup>8</sup> and Takaya<sup>9</sup> showed that supernumerary limbs could be induced from the flanks of newts by implantation of fragments of nasal placode tissue. Nearly all these supernumeraries were single and of reversed anteroposterior polarity with respect to the body. This is just what would be expected if the placode induces a new zone of competent tissue posterior to the host polarising region.

Considering more recent work, it is clear that the

existence of a posterior polarising region makes amphibian limb development seem much more like that of other vertebrates than previously thought. A polarising region has been described in the limb buds of the chick and mouse with very similar properties<sup>9-12</sup>. There remain two important differences, however: first, the interaction in *Ambystoma* and *Pleurodeles* seems to occur several days before the formation of a visible limb bud, whereas that of the chick is active during bud outgrowth; and second, the urodele limb rudiment is highly regulative in the anteroposterior axis whereas that of the chick is not.

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## Langerhans cells form a reticuloepithelial trap for external contact antigens

THE outermost covering of the body, the epidermis, is a thin epithelial sheath comprising three distinct cell types, keratinocytes, melanocytes and Langerhans cells. The physicochemical and photo-protective role of the first two is well known, but the function of the third is still unknown, over a hundred years since its discovery<sup>1</sup>. The Langerhans cell was reported earlier as having a sensory role, or as being an effete form of the dendritic melanocyte below it<sup>2</sup>, but it occurs in the dermis<sup>3</sup> and is mesenchymal, rather than neuroectodermal, in origin. Its fine structure reveals no melanin, but distinctive racquet-shaped granules. It has also been proposed that it is a macrophage<sup>4</sup> or that it may be involved in keratinisation, but recent evidence has suggested an immune function for the Langerhans cell in the development of contact dermatitis<sup>5,6</sup>. To investigate this possibility, we have exposed separated epidermal sheets to metals, aldehydes and amines known to act as contact antigens, and have observed their distribution histologically. We report here the selective uptake of antigens causing allergic contact dermatitis by Langerhans cells of the epidermis in guinea pigs and man.

Adult male white Hartley guinea pigs (300 g) were killed by cervical dislocation. After electric clipper removal of hair, ears were excised and inner and outer skin layers removed from cartilage by forceps. Human specimens were superficial horizontal skin slices removed by razor blade, with or without local lidocaine anaesthesia from male and female white adults with normal skin. The epidermis was finely dissected free after incubation in buffered EDTA solution for 2 h 37 °C (ref. 7), or in 1 M sodium bromide (pH 6.8, 0.1 M phosphate buffer) for 30 min. To brace metal antigens, the epidermal sheets were kept in mercuric chloride (7 mM), nickel chloride hexahydrate (12 mM), cobaltous chloride hexahydrate (12 mM), or chromic chloride hexahydrate (12 mM) in Tris-maleate buffer, pH



7.4 with 5% glucose for 60 min at 37 °C. The sheets were developed by immersion in 175 mM ammonium sulphide for 20 min at room temperature (22 °C), and then fixed in cold cacodylate formaldehyde (4 °C) for 20 min. This was followed by a 4-min exposure to Timm silver nitrate developer. Control staining of the Langerhans cells used lead nitrate (6 mM) in adenosine-5-triphosphate (ATP), glucose, Tris-maleate buffer with MgSO<sub>4</sub> added. The specimens were mounted in glycerine jelly.

The uptake of formaldehyde and glutaraldehyde was studied by exposing the separated epidermis to formaldehyde or glutaraldehyde (4%) solution for 20 min at 4 °C followed by paraphenylenediamine (PPD) (46 mM) in

cells could be seen clearly only when fresh epidermal shavings were used, and it was difficult to achieve uniform staining. All the results were essentially the same in guinea pigs as in man.

This study reveals a hitherto unknown affinity of the Langerhans cell network for the most commonly known external contact sensitizers and indicates that haptens or antigens which penetrate the stratum corneum into the living avascular epidermis are trapped by a specialised protective reticulum of dendritic cells. We call this network of Langerhans cells a reticuloepithelial system, indicating its close relationship to the reticuloendothelial system which clears the body of antigens which have entered the general circulation. Thus, the epidermis has a trap for external contact antigens which parallels that of the lymph node and spleen for circulating antigenic material. The cells which remove foreign antigens from the epidermis are metalophilic, as are the specialised non-phagocytic reticuloendothelial cells of the spleen<sup>8</sup>. The Langerhans cells are further characterised by a high level of ATPase activity typical of reticuloendothelial cells<sup>9</sup>. Even more striking is the dendritic nature of the Langerhans cell, which duplicates that of the antigen-retaining cells of the lymph nodes and spleen<sup>10</sup>. These dendrites help the cell in its protective and clearing functions and in well stained specimens they form a virtually impassable intercellular system high in the epidermis.

The anatomy of the epidermis does not require classical phagocytes, since it is molecular, rather than particulate, matter which enters this syncytium. The Langerhans cell is thus a specialised mesenchymal scavenger which engulfs and processes chemicals entering the epidermal intercellular spaces from above or below. It must metabolise many compounds by its highly active intracellular enzyme system<sup>4</sup>, but other compounds such as we have studied persist as sensitising antigens localised to the Langerhans cell. This cell seems to be the target cell for cytotoxicity and immunopathology in contact dermatitis and could be a new model for observations on the nature of delayed hypersensitivity. Our investigation was limited to antigens which could be identified by direct histological techniques, but it should be possible to trace other antigens to this reticuloepithelial system by appropriate radioactive and immunofluorescent techniques. In any event, the monoplanar topography of the Langerhans cell system within the upper epidermis may make it a more attractive test model for study than the analogous antigen-trapping cell system hidden within the spleen, lymph node and blood vessels.

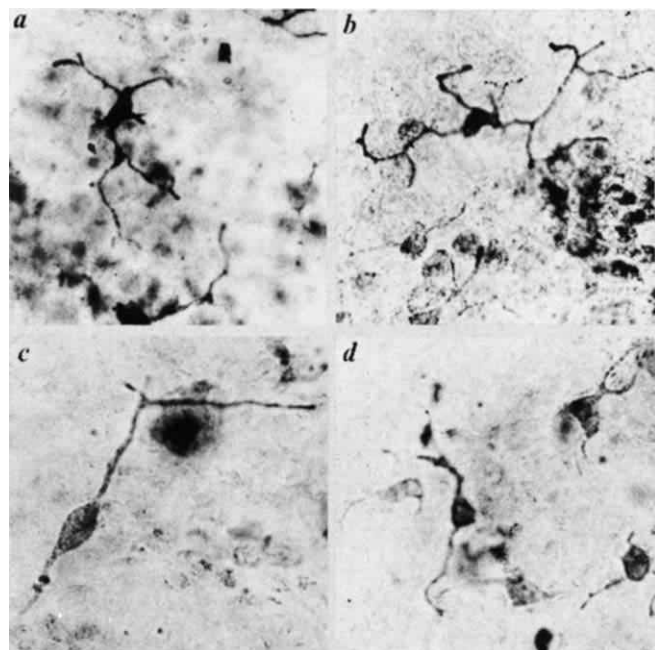
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**Fig. 1** Demonstration of selective uptake of various antigens by Langerhans cells in human epidermis. *a*, Mercuric chloride (ammonium sulphide developer, formaldehyde fix, Timm intensifier); *b*, nickel chloride (as in *a*); *c*, chromic chloride (as in *a*); *d*, formaldehyde (paraphenylenediamine developer, hydrogen peroxide intensifier). ( $\times 352$ .)

sodium borate-carbonate buffer (pH 10.8) for 60 min. Subsequent exposure to 3% hydrogen peroxide for 10 min at room temperature (22 °C) preceded mounting in glycerine jelly.

Amine uptake was studied by exposing fresh, unfixed epidermal razor biopsy specimens to PPD (46 mM) in borate buffer (pH 10.8), or to ethylenediamine dihydrochloride (8 mM) in 5% glucose in water for 20 min at 37 °C. The PPD-treated epidermis was then exposed to 5% *o*-phthalaldehyde in absolute ethanol for 10 min at room temperature and mounted in xylene. The ethylenediamine-treated epidermis was exposed to 5% *o*-phthalaldehyde in xylene for 10 min at room temperature and mounted in xylene.

All the antigens listed were selectively taken up by high level dendritic cells which could be identified in controls as Langerhans cells by the standard ATPase stain<sup>7</sup>. The metallic antigens, mercury, nickel and chromium (Fig. 1*a-c*) produced particularly clear outlines of the cell body with long dendrites coursing between the adjacent keratinocytes. Cobalt deposition was equally specific, although the dendrites here appeared shorter and less filamentous.

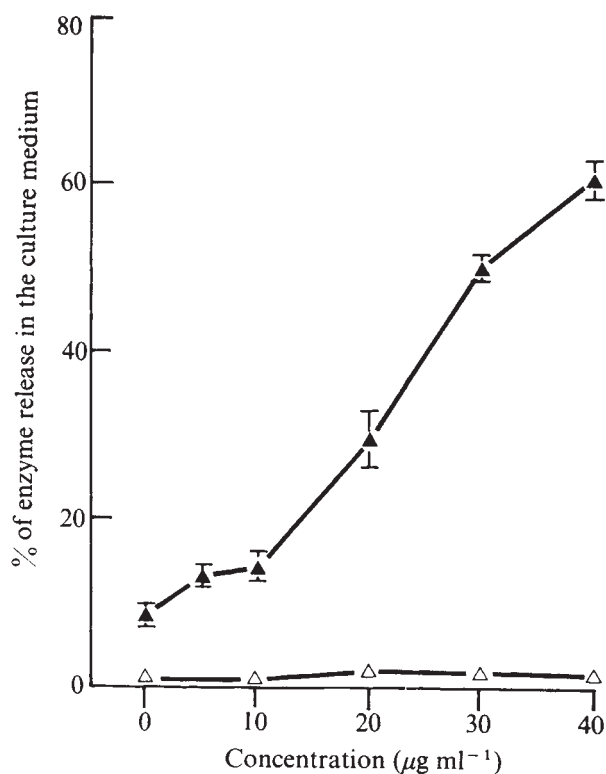
The uptake of formaldehyde clearly delineated the high level dendritic cell (Fig. 1*d*), as did glutaraldehyde. The uptake of PPD and ethylenediamine by the Langerhans



## Ability of activated complement components to induce lysosomal enzyme release from macrophages

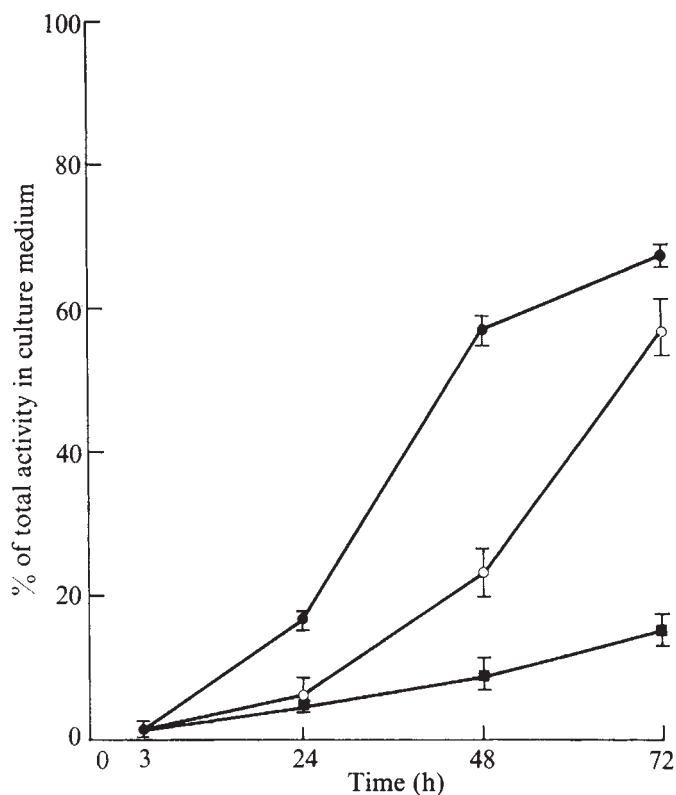
THE mechanism underlying chronic inflammation is of considerable medical importance both in temperate countries—where diseases such as rheumatoid arthritis are common—and in tropical countries—where various chronic inflammatory conditions cause much disease and disability. It is generally accepted that the macrophage is the central cell in chronic inflammation. We have drawn attention to the parallelism between the capacity of various agents to induce chronic inflammation *in vivo* and selective lysosomal enzyme secretion from cultures of macrophages<sup>1,2</sup>. The mechanism by which macrophage enzyme secretion is induced is unknown: we now report a mechanism mediated by cleavage products of the complement component C3.

Mononuclear phagocytic cells interact with complement in several ways. Human monocytes, mouse peritoneal macrophages and rabbit alveolar macrophages have receptors for C3b (refs 3 and 4). The experiments reported here show that attachment of C3b to mouse macrophages in culture results in a dose-dependent and time-dependent release of lysosomal enzymes into the medium. Figures 1 and 2 show data for  $\beta$ -galactosidase and  $\beta$ -glucuronidase, and similar observations have been made with *N*-acetyl- $\beta$ -D-glucosaminidase. The selectivity of the release, which



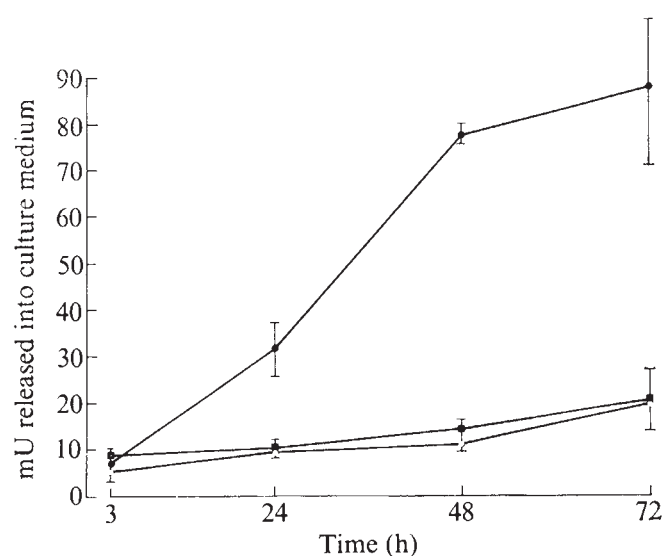
**Fig. 1** The release of  $\beta$ -galactosidase ( $\blacktriangle$ ) and lactate dehydrogenase ( $\triangle$ ) from macrophages exposed to increasing concentrations of C3b. Mouse peritoneal macrophage cultures were prepared as before<sup>12</sup>. C3b was generated and purified as before<sup>13</sup>. Cells were exposed to increasing amounts of C3b for 72 h. At this time the culture medium was removed and the adherent cells were washed once with phosphate-buffered saline and then released by adding saline containing 0.1% (w/v) Triton X-100 (BDH). All fractions were assayed for  $\beta$ -galactosidase activity by the method of Conchie *et al.*<sup>14</sup>. Lactate dehydrogenase was assayed by determining the rate of oxidation of reduced nicotinamide adenine dinucleotide at 340  $\mu$ m. Each value represents the mean  $\pm$  s.d. of four separate plates.

occurs without cell killing, is shown morphologically and by the failure of lactate dehydrogenase to appear in the medium; the levels of this enzyme increase in cells to which C3b is attached. The C3a fragment induces release of histamine from cells that store this substance<sup>5</sup>. We have observed that addition of C3a to macrophage cultures also results in release of acid hydrolases. But when the time course was studied, lysosomal enzyme release in the presence of C3a was always accompanied by loss of cellular lactate dehydrogenase (Figs 2 and 3), showing that the cells had been killed. The distinct effects of C3b and C3a on mononuclear phagocytes *in vitro* are interesting in view of the possible differences in the ways by which they interact with membranes.



**Fig. 2** The time dependence of macrophage  $\beta$ -glucuronidase release induced by the purified complement components C3 ( $\blacksquare$ ), C3b ( $\circ$ ) and C3a ( $\bullet$ ). Highly purified guinea pig C3 was prepared as before<sup>15</sup>. For generation and purification of C3a, purified C3 was incubated with the C3-cleaving complex which is formed when cobra venom factor interacts with factor B, factor D and  $Mg^{2+}$  (ref. 16). This reaction mixture was put over a column of Sephadex G-100 (Pharmacia). The fractions which mediated contraction of isolated terminal guinea pig ileum were pooled and concentrated. Purified C3, C3b and C3a were provided by Dr D. Bitter-Suermann, Institut für Medizinisch Mikrobiologie, Mainz. The experimental conditions were identical to those described in Fig. 1. Each point represents the mean  $\pm$  s.d. of four separate plates.

It is now clear that both granulocytes<sup>6,7</sup> and macrophages, phagocytic cells associated with acute and chronic inflammation respectively, can selectively release lysosomal hydrolases through the mediation of complement cleavage products, with no detectable loss of viability. This phenomenon may explain the ability of macrophages to cause tissue damage and degradation at sites of chronic inflammation while retaining their viability over long periods. This interpretation is supported by evidence that other agents inducing chronic inflammation, such as group A streptococcal cell walls<sup>8</sup>, dental plaque<sup>9</sup>, carrageenan<sup>1,2</sup> and mouldy hay dust (our unpublished results with J. Edwards), also cause selective release of lysosomal hydrolases from viable macrophages *in vitro*. All these agents



**Fig. 3** Time-dependent release of lactate dehydrogenase from macrophages exposed to the purified complement components C3 (○), C3b (■) and C3a (●). The experimental conditions were identical to those described for Figs 1 and 2. Each point represents the mean  $\pm$  s.d. of four separate plates.

have been shown to activate complement by the alternative pathway<sup>10,11</sup>, so generating C3b, which may be the common factor mediating enzyme release from macrophages. This system is amplified because we have found that enzymes released from stimulated macrophages can cleave C3, generating more C3b, which would, in turn, induce further enzyme release. The role of this amplification system in chronic inflammation clearly merits detailed study.

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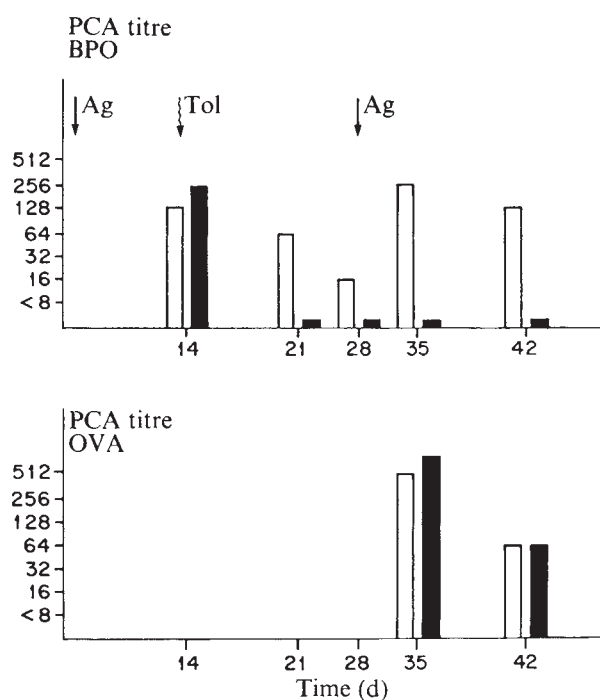
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## Isologous IgG-induced tolerance to benzyl penicilloyl

INDUCTION of tolerance by haptens linked to non-immunogenic carriers<sup>1–5</sup> is used widely, not only to investigate the cellular mechanism of tolerance<sup>6</sup> but also as a new approach

to the treatment of experimental immune disease<sup>7</sup>. A potentially therapeutic application of carrier-induced tolerance is the treatment of allergic diseases. In this respect, systemic hypersensitivity reactions to penicillin constitute a major cause of death due to anaphylaxis, and penicilloyl determinant is involved in approximately half of the cases<sup>8–10</sup>. We report here the suppression of reaginic antibody to the benzyl penicilloyl (BPO) determinant in unprimed as well as primed mice.

Benzyl penicilloyl was bound covalently in the penicilloyl configuration to several protein carriers, and residual unbound hapten was removed by gel filtration<sup>11</sup>. IgG1 (MOPC245) mouse gamma globulin (MGG1) was prepared as before<sup>12</sup>. The following hapten carrier conjugates were used: BPO<sub>17</sub>-ovalbumin (OVA) as immunising antigen, BPO<sub>23</sub>-MGG1 as the tolerogen and BPO<sub>37</sub>-bovine gamma globulin (BGG) as challenge antigen in the 4-h passive cutaneous anaphylaxis assay in Sprague-Dawley rats for mouse IgE antibody<sup>13</sup>.

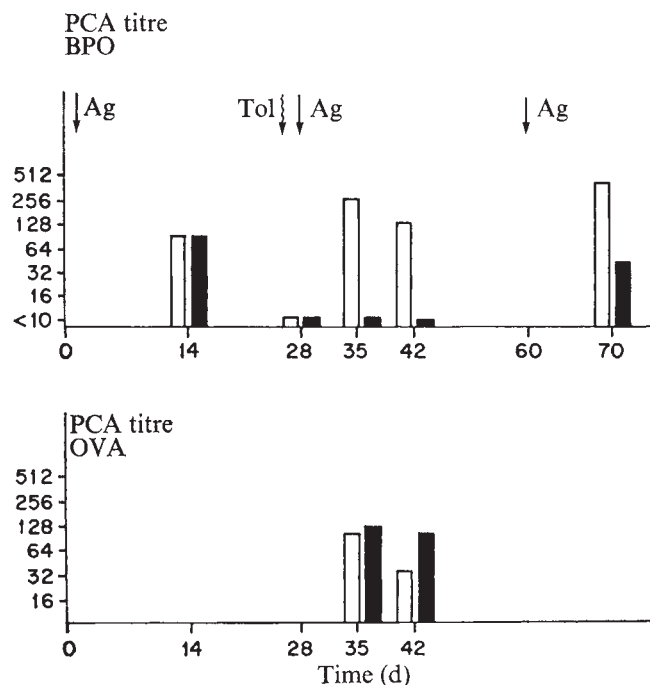


**Fig. 1** Suppression of BPO IgE antibody in BDF<sub>1</sub> mice with BPO-MGG1 during an ongoing primary immune response. Each column represents the BPO and OVA PCA titre of pooled sera from either 10 control mice or 10 mice treated with tolerogen (Tol). The small arrows indicate the time of tolerogen or antigen administration. Open columns, control; black columns, treated with tolerogen.

The immunising regimen of Levine and Vas<sup>14</sup> was used to raise reaginic anti-hapten antibody in (C57BL/6 × DBA/2)F<sub>1</sub> (BDF<sub>1</sub>) mice. To determine whether the primary immune response to BPO could be suppressed by a single dose of tolerogen, a group of 10 (6–8-week-old) male BDF<sub>1</sub> mice were given 1 mg of BPO<sub>23</sub>-MGG1 intravenously. Immediately thereafter these mice, as well as a group of 10 control BDF<sub>1</sub> mice, were injected intraperitoneally with 1  $\mu$ g of BPO<sub>17</sub>-OVA and 1 mg of Al(OH)<sub>3</sub> in 0.5 ml of phosphate-buffered saline (pH 7.4). The mice were bled on day 14 (which was shown in preliminary studies to correspond to the peak of the primary IgE antibody) and on days 21 and 28. After the third bleeding, both groups were again injected with BPO<sub>17</sub>-OVA and alum and were bled 4 and 8 d later. The serum from individual mice was pooled to obtain sufficient for passive cutaneous anaphylaxis (PCA) titration. The titre of IgE anti-BPO antibody for the control group on day 14 was 1/128, 1/32 on day 21 and not detectable

in serum diluted 1:8 on day 28. In contrast, in the group treated with tolerogen, no IgE antibody to BPO was detectable in serum obtained on days 14, 21 and 28 at a dilution of 1:8. The secondary response to BPO was also suppressed in the animals made tolerant to BPO-MGG1. The BPO PCA titre was 1/128 on day 4 and 1/2,048 on day 8 for the controls after the second immunogen injection. On both days, no BPO IgE antibody was detectable in serum diluted 1:8 in animals previously rendered tolerant.

To determine whether pre-existing IgE antibody response to BPO could be suppressed, two groups of 10 BDF<sub>1</sub> mice were immunised as described above. After 14 d both groups had IgE anti-BPO antibody in their serum (Fig. 1). At this time, one group of mice received 2.5 mg of BPO<sub>23</sub>-MGG1 intravenously. All mice were bled 21 and 28 d after the initial immunisation. Both groups were subsequently injected with 1 µg of BPO<sub>17</sub>-OVA in Al(OH)<sub>3</sub>, and bled 1 and 2 weeks later. Figure 1 shows that the reaginic antibody to BPO was suppressed during both the primary and secondary response. In the secondary response, the suppression to BPO was hapten specific, since the response to the carrier OVA was unaffected.



**Fig. 2** Suppression of BPO IgE antibody in BDF<sub>1</sub> mice with BPO-MGG1 in the secondary immune response of primed mice. Each column represents the BPO and OVA PCA titre of pooled sera from either six control mice or six mice treated with tolerogen. The small arrows indicate the time of tolerogen and antigen administration. Columns as in Fig. 1.

Finally, tolerance induction was studied in mice which had been primed with antigen after the IgE response had subsided. Immunised animals, given 1 mg of tolerogen before secondary immunisation with BPO<sub>17</sub>-OVA, did not have detectable IgE anti-BPO antibody in their serum during the secondary response. In contrast, the IgE antibody response to the carrier OVA was the same in both groups (Fig. 2). After the third immunisation with antigen, the response to BPO was also diminished in animals treated with tolerogen before the second immunisation. We believe we were dealing primarily with IgE Ab because the method of immunisation favours the production of IgE<sup>14</sup>. Mouse IgE but not IgG1 primed rat mast cells for PCA reaction<sup>13</sup> and finally the antibody involved in our experiment was susceptible to heating at 56 °C for 4 h (ref. 13).

The results suggest that the formation of reaginic antibody to the major determinant of penicillin can be suppressed in mice by injection of BPO isologous IgG before primary immunisation, during a continuing primary immune response, and before a secondary injection of hapten conjugate into primed animals. Suppression of the response was specific for the hapten, since IgE antibody to the carrier OVA was unaffected. The lack of fatal reactions in sensitised mice given 2.5 mg of BPO<sub>23</sub>-MGG1 intravenously is interesting. The dose administered may constitute antigen excess in this system, and/or the presentation of the hapten on isologous Ig may have a protective effect. Katz and Benacerraf, using DNP-D-GL as a tolerogen<sup>15,16</sup>, and Lee and Sehon, using DNP-MGG as a tolerogen<sup>17,18</sup>, have shown that IgE antibody to DNP can be suppressed in either primed or unprimed rodents. Our studies extend these observations to an antigenic determinant which is prominently involved in human hypersensitivity reactions. The potential use of autologous human IgG as a carrier for induction of tolerance to antigenic determinants responsible for allergic diseases may have interesting implications.

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## Retransformation of a thermosensitive BALB/c-3T3 transformant by murine sarcoma virus at the non-permissive temperature

CERTAIN fibroblast cell lines exhibit some of the properties of transformed cells at one temperature while retaining normal properties at a higher temperature. For example, the spontaneous transformants of BALB/c-3T3 cells isolated by W. Eckhart (unpublished results) can grow in soft agar at 32 °C (the permissive temperature) but not at 39 °C (the non-permissive temperature). They show trans-



formed morphology at 32 °C but normal morphology at 39 °C (ref. 1). They have certain temperature-sensitive surface properties such as ability to grow in Agarose, loss of a transformation-sensitive iodinated glycoprotein (LETS)<sup>2,3</sup>, increase in total cell proteolytic activity<sup>4</sup> and loss of the growth promoting activity of fibroblast growth factor<sup>5</sup>. But they behave normally with respect to the regulation of intracellular events. Unlike transformed fibroblasts, both at permissive and non-permissive temperatures, BALB/c-3T3 cells exhibit density-dependent growth inhibition<sup>6</sup>. They cease growing as a result of serum limitation and express the typical increases in protein synthesis, ribosomal RNA synthesis and DNA synthesis when growth is

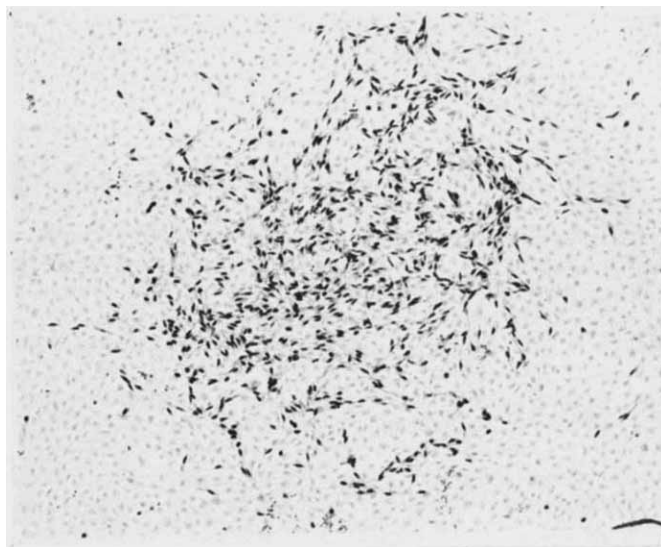
**Table 1** Transformation assay of tsCl.1 and BALB/c-3T3 Cells with MSV at 39 °C

| Virus dilution   | No. of transformed foci per plate |            | No. of labelled foci per plate |            |
|------------------|-----------------------------------|------------|--------------------------------|------------|
|                  | tsCl.1                            | BALB/c-3T3 | tsCl.1                         | BALB/c-3T3 |
| 10 <sup>-2</sup> | ≤500                              | ≥500       | ≥500                           | ≥500       |
| 10 <sup>-3</sup> | 150±20                            | 87±12      | 185±24                         | 105±15     |
| 10 <sup>-4</sup> | 17±5                              | 10±3       | 25±8                           | 13±4       |
| Mock infected    | 0                                 | 0          | 0                              | 0          |

tsCl.1 or BALB/c-3T3 cells (10<sup>6</sup>) were plated per 90-mm dish in Dulbecco's modified Eagle's medium containing 10% foetal calf serum (Flow). A day later, the cells were treated in 1 ml of medium containing polybrene (25 µg ml<sup>-1</sup>) and Kirsten MSV (10<sup>6</sup> FFU) at the appropriate dilutions, or in mock-infected controls, for 2 h with frequent shaking. The cells were then allowed to incubate in medium containing 10% serum. Transformed foci could be seen 3–4 d after exposure to virus and were scored at 7 d. Results obtained using a microscope, are expressed as the number of transformed foci per plate. Alternatively, on day 7, when the cultures became confluent, the cells were incubated in medium containing 1% serum and 3-<sup>3</sup>H-thymidine (3 µCi ml<sup>-1</sup>) at 1 µM for 24 h. Subsequent fixing and autoradiography of the dishes showed that only cell nuclei of transformed foci became labelled. Dishes were stained with Giemsa and results were expressed as the number of visible radioactively labelled colonies per dish. All numbers represent averages of three separate determination ± two standard deviations.

initiated<sup>1,6</sup>. The evidence therefore suggests that the transformants have lost their potential to express a transformed phenotype at the non-permissive temperature. I now show that this is not an irreversible loss by demonstrating that murine sarcoma virus (MSV) can transform the cells at the non-permissive temperature.

An independent temperature-sensitive clone of BALB/c-3T3 cells (tsCl.1) was infected at 39 °C with a Kirsten strain of MSV (supplied by Dr G. S. Martin). Transformed foci appeared in both tsCl.1 and the control BALB/c-3T3<sup>7</sup>-infected cultures. Both mutants and parent cell cultures were characterised with respect to saturation densities, regulation of DNA synthesis, growth in agar, and proteolytic



**Fig. 1** Radioactively labelled focus of BALB/c-3T3 cells induced by MSV at 39 °C. Seven days after infection with MSV, the cells were exposed to <sup>3</sup>H-thymidine (3 µCi ml<sup>-1</sup>) at 1 µM in Dulbecco's modified Eagle's medium containing 1% foetal calf serum for 24 h and processed as described previously. Cells were stained with Giemsa and photographed under the light microscope (×35).

activity in a casein overlay assay. The data presented here indicate that tsCl.1 can be transformed at 39 °C and that it can be rendered as fully transformed as the virally transformed cell lines Py3T3 (ref. 8) and SV3T3 (ref. 9).

Logarithmically growing cells were exposed to various dilutions of Kirsten MSV (10<sup>6</sup> FFU) at 39 °C. Foci were seen 3–4 d after infection but were routinely counted on day 7. As reported previously<sup>10–12</sup>, their detection was difficult and required optimal culture conditions, such as rich medium and high inoculation densities. Transformed foci appeared as round refractile cells, adhered poorly to the plastic dish, had a tendency to float off into the medium and could not be stained easily. To screen large numbers of cultures for transformed foci rapidly, I developed a method which involves exposure of the virus-infected cultures to <sup>3</sup>H-thymidine in a medium containing low serum concentrations (0.5–1% foetal calf serum). In confluent cultures, transformed foci showed incorporation of <sup>3</sup>H-thymidine into labelled nuclei, whereas the uninfected parental BALB/c-3T3 cells remained unlabelled. As Fig. 1 shows, after autoradiography<sup>13</sup> and subsequent staining of the dishes, transformed foci could be recognised and counted. Table 1 shows the transformation assay of tsCl.1 and the parental

**Table 2** Caseinolytic activity and growth in soft agar

| Cell type  | Temperature (°C) | Degree of casein hydrolysis (scale 0 to +4) |            | Colony formation (%) |            |
|------------|------------------|---------------------------------------------|------------|----------------------|------------|
|            |                  | MSV infected                                | Uninfected | MSV infected         | Uninfected |
| tsCl.1     | 32               | +4                                          | +3         | 34                   | 22         |
|            | 39               | +4                                          | 0          | 39                   | 0.7        |
| BALB/c-3T3 | 32               | +3                                          | 0          | 20                   | 0.3        |
|            | 39               | +3                                          | 0          | 28                   | 0.5        |

Ten transformed foci were selected independently from each of the MSV-infected tsCl.1 and BALB/c-3T3 cells at various dilutions. While the uninfected parental cultures contained no detectable virus, all focus-derived lines showed evidence of virus production as determined by an XC plaque assay<sup>11</sup>, thus excluding spontaneously transformed foci. For the measurement of caseinolytic activity, untransformed BALB/c-3T3, mutant tsCl.1, and cloned MSV-transformed BALB/c-3T3 and tsCl.1 cells grown at their respective temperatures were each plated at 10<sup>5</sup> cells per 5-cm Petri dish in Dulbecco's modified Eagle's medium supplemented with 10% foetal calf serum. After 5 h the monolayers were washed twice in serum-free medium and overlaid with 1 ml of medium at 45 °C, containing 0.6% Agarose (Difco), 2% powdered milk (Marvel), and 0.5% green African monkey serum (Gibco). The Agarose was allowed to harden at room temperature and the plates were then returned to incubate at 32 °C and 39 °C. The extent of plasmin-mediated caseinolysis was estimated after 40–50 h at 39 °C and after 80–120 h at 32 °C, based on a scale of 0 (no clearing) to +4 (complete clearing), according to the proportion of casein removed. The ability of the cells described above to grow in soft agar was determined by plating 10<sup>5</sup> cells in medium containing 10% foetal calf serum in 5-cm Petri dishes. A day later cells were overlaid with 5 ml of a suspension of 0.33% agar (Difco) and 10% foetal calf serum in medium at 45 °C. Cells were incubated at 32 °C or 39 °C and the number of colonies per dish determined 2 and 3 weeks later. The percentage of cells which grew in agar is shown. Results represent averages of the focus-derived lines as determined in three separate experiments.

BALB/c-3T3 cells at 39 °C. Transformed foci in the unstained dishes were scored, with the aid of a microscope, 7 d after infection. They were then compared with the numbers of labelled foci in the autoradiographed and stained dishes. The use of labelled nuclei makes possible detection of small colonies so that the numbers of labelled foci were slightly higher. The results showed essentially a one-hit response curve<sup>12</sup>.

Fibroblasts transformed by DNA or RNA tumour viruses show markedly higher levels of proteolytic activity than do their normal untransformed counterparts<sup>14,15</sup>. This arginine-specific protease converts serum plasminogen to its enzymatically active form, plasmin. The plasmin in turn can be detected by its ability to hydrolyse milk protein in a casein overlay assay<sup>16</sup>. MSV-transformed tsCl.1 and BALB/c-3T3 foci were selected independently and grown at their respective temperatures. Monolayers of the cloned cultures and their normal counterparts were overlaid with Agarose containing casein in the presence of 0.5% monkey serum, and the extent of caseinolysis was measured at 32 °C and 39 °C. Thus, the transformed tsCl.1 and BALB/c-3T3 cells showed a marked caseinolytic activity over the non-transformed counterparts at 39 °C (Table 2). Similarly, when the cultures were trypsinised and tested for their ability to grow in soft agar<sup>17</sup>, another parameter for measuring the degree of transformation in cell lines, the MSV-transformed variant and parent cells formed 39% and 28% colonies at 39 °C, respectively, compared with 0.7% and 0.5% colony formation of the uninfected cultures (Table 2).

Cloning efficiency and saturation density of the MSV transformants were higher at both temperatures compared with the uninfected cell counterpart at 32 °C. Thus, the final cell density of tsCl.1 in medium containing low serum concentration (1% foetal calf serum) was approximately twice as great at 32 °C than at 39 °C ( $2 \times 10^6$  and  $0.9 \times 10^6$  cells per 5-cm dish at 32 °C and 39 °C, respectively), similar to untransformed 3T3 cells. In contrast, approximately a tenfold increase in saturation density of the MSV-transformed tsCl.1 and BALB/c-3T3 cells was observed over the normal phenotype. At low serum concentrations, MSV-transformed tsCl.1 cells reached saturation densities of  $19 \times 10^6$  cells per 5-cm dish at 39 °C and 32 °C, respectively. Similarly, MSV-infected tsCl.1 cultures left to become confluent and exposed to <sup>3</sup>H-thymidine for 24 h at 39 °C and for 48 h at 32 °C showed incorporation of radioactivity only into nuclei of transformed foci. This was also the case for the parental BALB/c-3T3 cells (Fig. 1), which is another method of demonstrating that the MSV-transformed cells have overcome density-dependent inhibition of growth with respect to DNA synthesis.

Whether retransformation of a temperature-sensitive cell mutant at the non-permissive temperature is a general phenomenon that can be carried out with either DNA or RNA viruses remains to be determined. Renger retransformed temperature-sensitive SV3T3 cells with MSV at the non-permissive temperature<sup>18</sup> but the same cells could not be retransformed with high multiplicity SV40 (ref. 19). Similarly, SV3T3 revertants initially isolated by Pollack *et al.*<sup>20</sup>, and the revertants of polyoma-transformed BHK cells<sup>21</sup> could not be retransformed by their respective viruses but could be rendered fully transformed by exposure to murine<sup>18</sup> or hamster sarcoma<sup>21</sup> viruses, respectively. In this study, however, tsCl.1 and another similar independent clone tsCl.10 (supplied by Dr W. Eckhart) could be retransformed both by an RNA virus (MSV) and by a DNA virus (SV40) at the non-permissive temperature. Unlike SV3T3, the SV40-transformed ts clones could grow in soft agar (not shown).

In summary, MSV-induced transformation of temperature-sensitive cell variants can be accomplished at the non-permissive temperature. Thus, I have demonstrated growth in soft agar, increase in caseinolytic activity, higher satura-

tion densities and loss of regulation of growth and DNA synthesis after MSV transformation. My results suggest that a virus function can render these temperature-sensitive cells fully transformed with respect to certain properties of their intracellular machinery. Alternatively, the process of virus infection may induce a cell regulatory function. This possibility is being investigated, using a temperature-sensitive mutant of MSV to determine whether complementation can occur between the cellular mutation and the viral mutations which render the viral transformation temperature sensitive.

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## Exceptional mutagenicity of a benzo[a]pyrene diol epoxide in cultured mammalian cells

BENZO[a]PYRENE (BP) is one of a group of chemically inert carcinogens which, in order to exert their cytotoxic, mutagenic and carcinogenic effects, require metabolic activation by mixed function oxygenases (MFO) to products capable of covalent reaction with cellular macromolecules<sup>1-3</sup>. More specifically, it has been shown in mouse skin<sup>4</sup> and various cultured mammalian cells<sup>5</sup>, that, in the case of DNA, the extent of this reaction correlates with the carcinogenic potency of the compound. The hypothesis that DNA is the important target for this series of hydrocarbons is further supported by evidence that the most carcinogenic members are also the most mutagenic towards hamster cells *in vitro*<sup>1</sup>. Here we present evidence that a diol epoxide derivative of BP is a powerful mutagen when applied to mammalian cells, and suggest therefore that this compound is the ultimate carcinogenic metabolite of BP.

Although various models have been proposed regarding the nature of the BP-DNA reaction<sup>6</sup>, convincing experimental evidence has been lacking. Recent chromatographic analysis of DNA from hamster embryo cells exposed to <sup>3</sup>H-BP, however,



has established that, for this hydrocarbon, the binding species is a *trans*-7,8-dihydro-7,8-dihydroxy-9,10-epoxy derivative produced by a two-step metabolic event through the 7,8-epoxide<sup>7</sup>. Hulbert has predicted<sup>8</sup>, on theoretical grounds, that two stereoisomers of this diol epoxide are possible (designated syn and anti, see Fig. 1) and that only one of these, syn, would be expected to react significantly with DNA *in vivo*. Both isomers have been synthesised and the stereochemistry of each confirmed by analysis of their integrated proton nuclear magnetic resonance (NMR) spectra<sup>9</sup>.

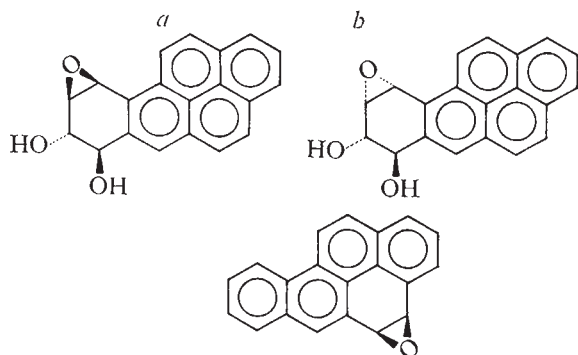


Fig. 1 Structural formulae of the three benzo[a]pyrene (BP) derivatives under investigation. *a*, BP diol epoxide syn configuration; *b*, BP diol epoxide anti configuration; *c*, BP-4,5-oxide (K-region epoxide). The two stereoisomers of BP diol epoxide have been designated syn and anti<sup>9</sup> to indicate the relationship of the epoxy group *cis* or *trans* to the 7-hydroxyl function. Hulbert<sup>8</sup> predicted that hydrogen bonding between these two groups, which is possible only in the case of syn, will facilitate opening of the epoxide, in effect producing an aralkyl carbonium ion on C9 or C10 of the molecule. This isomer would therefore react with appreciable  $S_N1$  character and so show little preference *in vivo* for strong nucleophiles over relatively weak nucleophiles such as DNA. Anti and BP-4,5-oxide on the other hand, would be expected to react predominantly by an  $S_N2$  mechanism and be much less reactive towards DNA, especially in the presence of strong competing nucleophiles such as the SH groups of glutathione and proteins.

We therefore thought it prudent to examine, in cultured mammalian cells, the cytotoxic and mutagenic properties of both diol epoxide isomers and to compare their respective activities with BP-4,5-oxide, the so-called K-region epoxide. For this purpose we used V79 cells, an established line with undetectable MFO activity<sup>10</sup> and, as a mutational marker, resistance to the purine analogue 8-azaguanine. This system is used widely to study mutagens which act directly and it is generally accepted that variants arise as a consequence of gene mutation. Our results indicate that all three compounds differ in their ability to induce mutations at this locus and that one diol epoxide isomer has exceptional mutagenic properties.

Figure 2 summarises the results of several experiments in which the three derivatives were compared in identical conditions. Parallel cultures of exponentially growing cells were exposed for 24 h to a range of concentrations of each compound, and replicate samples were plated for determination of induced cytotoxicity and mutagenesis (details in Fig. 2 legend). For each derivative a similar type of multitarget dose-survival curve was obtained, with a shouldered region at low doses followed by a steep exponential portion. The dose range required to achieve this response, however, varied, approximately in the ratio 1:3:6 for anti, syn and BP-4,5-oxide respectively. Similarly, the corresponding  $D_0$  values were calculated as 0.10, 0.25, 0.85  $\mu\text{g cm}^{-3}$  of culture medium. Further interpretation of the survival data in terms of the extent of the hydrocarbon-DNA reaction was precluded because labelled BP diol epoxides were not available.

The mutational response, as a measure of specific DNA modification, not only reflected the overall variations observed in the survival experiments but also revealed more subtle

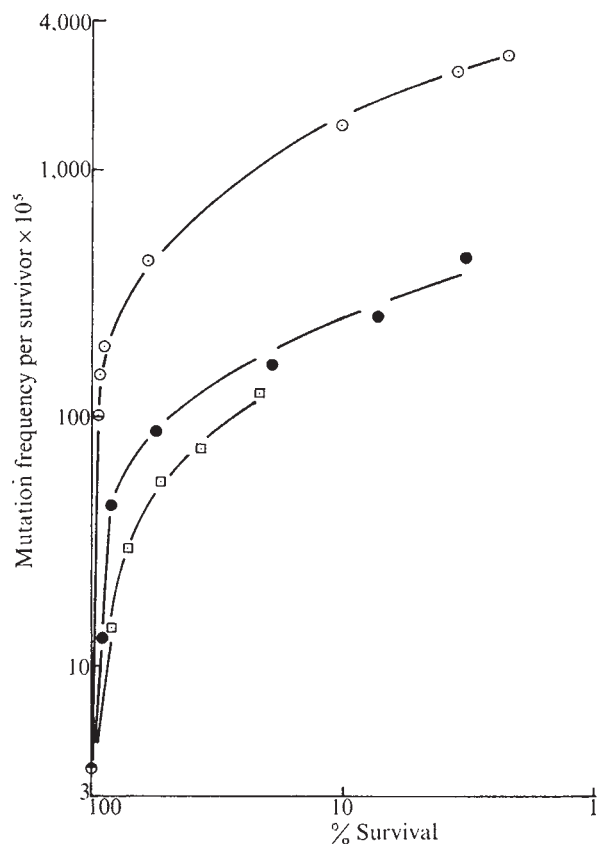


Fig. 2 Mutagenesis of V79 Chinese hamster cells as a function of percentage survival:  $\circ$ , BP diol epoxide anti;  $\bullet$ , BP diol epoxide syn;  $\square$ , BP-4,5-oxide. The results, derived from individual dose-response experiments, were expressed in this way to facilitate a direct comparison between the derivatives in terms of mutagenic efficiency. Mutation frequency was plotted on a logarithmic scale so that all three responses could be represented accurately on one graph. Linear-logarithmic plots of mutation frequency against survival respectively were linear below 85% survival. In all experiments 75-cm<sup>2</sup> plastic tissue culture flasks (Falcon), containing 30 cm<sup>3</sup> of Dulbecco's modified minimal essential medium with 10% foetal calf serum (Gibco), were each seeded with  $8 \times 10^5$  cells grown from a clonal stock frozen in liquid nitrogen. After 18 h the resulting exponentially growing cultures were treated with various doses of the compounds added in 0.1 cm<sup>3</sup> of dimethylsulphoxide (DMSO). All hydrocarbon solutions were made up immediately before use in DMSO which had been dried over a molecular sieve. The precise concentration of each derivative in solution was established by ultraviolet spectrophotometry. After 24 h of treatment at 37°C the cells were detached from each flask with trypsin-EDTA and the density of the resulting single-cell suspension was estimated with a haemocytometer. For the determination of induced toxicity,  $2 \times 10^5$  cells, and for mutagenesis  $5 \times 10^4$  cells, were plated in 5-cm and 9-cm plastic tissue culture dishes (Sterilin) respectively, and incubated at 37°C in a humidified atmosphere of 5% CO<sub>2</sub>:95% air. Mass selection of mutants defective in the purine salvage enzyme, hypoxanthine guanine phosphoribosyl transferase was effected by adding 8-azaguanine to the plates such that its final concentration in the medium was 30  $\mu\text{g cm}^{-3}$ . To achieve maximum recovery of mutants, a complete expression time curve<sup>11</sup> was constructed for each dose of hydrocarbon. In practice, five to ten replicates were treated with 8-azaguanine at each of six expression time points ranging from 0 to 120 h after plating. Survival plates were stained after 7 d of incubation, mutation plates 3 d later, and colonies of more than about 50 cells were scored macroscopically. Mutation frequencies were corrected for toxicity and calculated per surviving cell. The values shown in the figure are those obtained from the peak expression times which ranged from about 48 h for shoulder doses to 96 h for the more lethal treatments. Control frequencies never exceeded  $4 \times 10^{-5}$  per survivor. We have already discussed the various pitfalls encountered in this type of experiment<sup>12</sup>.



differences between the compounds. The dose response was cumulative for each compound, a characteristic observed by others who used these cells as targets for the classical mutagens EMS, MMS, X rays and ultraviolet light<sup>13,14</sup>. Although syn and BP-4,5-oxide proved relatively powerful mutagens, the absolute mutation frequencies obtained with anti were unexpectedly high. Wood *et al.* have tested BP-4,5-oxide in V79 cells<sup>15</sup> and the close agreement of our results with those for that epoxide served as a useful indication of the reproducibility of the system.

A double logarithmic plot of mutation frequency against survival is used frequently to discriminate between mutagens where the only measure of effective dose is induced toxicity. When the results discussed above are plotted in this way (Fig. 2) the true mutagenic potency of anti becomes clear, it being more than eight times as active as BP-4,5-oxide at 37% survival ( $e^{-1}$  = one lethal hit). This distinction between cytotoxic events and mutagenesis is best illustrated by applying the term "mutagenic efficiency"<sup>16</sup> to our data, which we define as the mean rate of increase of mutation frequency per lethal hit.

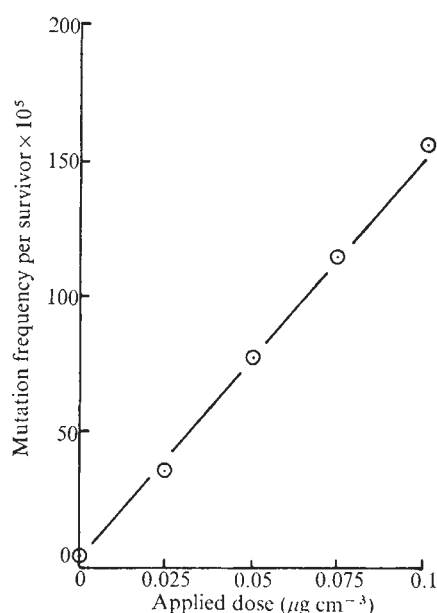


Fig. 3 The mutagenic activity of antiol epoxide at shoulder doses. Experiments were carried out as described in legend to Fig. 2.

For all three compounds linear-logarithmic plots of induced mutation frequency against survival respectively were approximately linear when only the exponential portion of each survival curve was considered, thus reflecting a direct proportionality between the two effects. The response in each case can therefore be represented adequately by an equation of the form:  $y = ax + b$ , where  $a$  is the slope and thus the mutagenic efficiency, and  $b$  is the intercept on the  $y$  (mutation frequency) axis, resulting from a higher rate of mutagenesis at minimally lethal and subtoxic doses. For anti, syn and BP-4,5-oxide these equations were calculated as:  $y_{\text{anti}} = 10^{-4} (56x + 20)$ ,  $y_{\text{syn}} = 10^{-4} (8.6x + 2.3)$  and  $y_k = 10^{-4} (8.6x + 0.1)$ , respectively.

Although BP-4,5-oxide was, not unexpectedly, the least powerful of the BP derivatives tested, the observed differences between the two diol epoxide isomers are difficult to explain on the basis of Hulbert's arguments<sup>8</sup>. Indeed, the syn isomer was predicted to be the most active. It should be appreciated, however, that mutagenesis has been examined at only a single genetic locus so far, and this may not be representative of the whole genome or indeed those targets relevant to carcinogenesis. Also, we have not finally established which isomer binds to DNA after BP is metabolised *in vivo*. Nevertheless, we feel that the possible contribution of these findings towards the understanding of BP carcinogenesis cannot be ignored.

Further examination of the anti isomer with respect to its mutagenic potency at sublethal shoulder doses proved equally exciting. The results (Fig. 3) demonstrate that in the absence of measurable toxicity, mutagenesis increases proportionally with dose. The type of low dose experiment is likely to be much more representative of human exposure to environmental BP. Moreover, the induction of cancer through somatic mutation(s) may well require that a powerful carcinogen be an efficient mutagen, that is that it can induce the necessary mutations in the DNA of the target cell with the minimum of concomitant side effects. The anti isomer of diol epoxide discussed here certainly meets this requirement.

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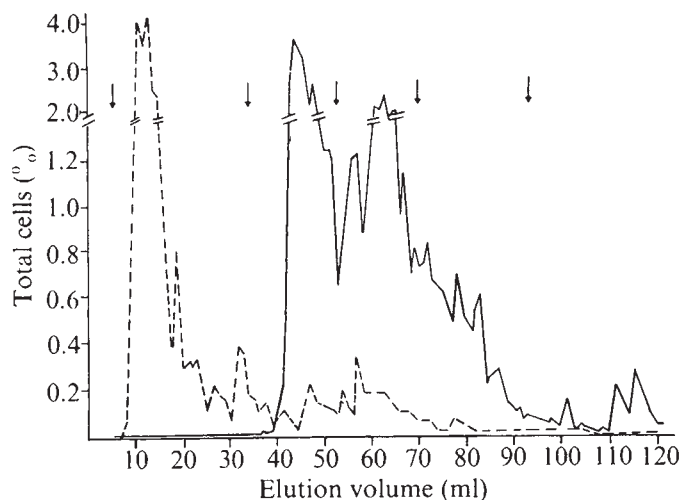
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## Tumorigenicity and the expression of cell-surface carbohydrates

TUMOUR cell populations are heterogeneous with respect to various biological properties<sup>1-3</sup>. Immunological properties that influence tumour-host relationships and metastatic properties of tumours may also differ between subpopulations of tumour cells<sup>4,5</sup>. In addition, conditions of growth may favour the emergence of subpopulations, and the immunogenic modification of susceptible subpopulations. We have reported that highly immunogenic tumour cells can be isolated from the parental tumour by concanavalin A (con A)-affinity chromatography<sup>6,7</sup>, and the relative proportion of different subpopulations varied with different conditions of growth. We now report that the tumorigenicity of murine adenocarcinoma varies as a function of the tumour subpopulations present, as defined by their expression of cell-surface carbohydrates.

The transplantable mammary adenocarcinoma A-10 (syngeneic to strain A/J mice) has been maintained as an ascitic tumour in B6AF<sub>1</sub> (C57BL/6  $\times$  A/J) mice, and in monolayer culture using RPMI 1640 medium, supplemented with 10% foetal calf serum and antibiotics.

The tumorigenicity of A-10 cells grown *in vivo* and *in vitro* is summarised in Table 1. An intraperitoneal inoculum of  $10^2$  or  $10^3$  cells grown *in vivo* routinely caused 100% mortality of B6AF<sub>1</sub> mice, whereas similar inocula of cells grown *in vitro* caused about 50% mortality. Inocula of up to  $10^6$  cultured cells were not always 100% fatal (J.J.K. and



**Fig. 1** The elution profile of A-10 cells from con A-affinity columns. The percentage of total cells is shown as a function of elution volume. This technique has been described in detail elsewhere<sup>7</sup>. Cells were prepared as described in Table 1, and eluted with the sequential addition (arrows) of PBS,  $\alpha$ -methyl-D-mannoside,  $\alpha$ -methyl-D-glucoside, sucrose and  $\alpha$ -D-glucose. ---, Cells grown *in vivo*; —, cells grown *in vitro*. Ammonium oxalate wash of cultured cells did not affect the elution profile.

W.A.S., unpublished results). This difference in tumorigenicity was probably mediated by immunological mechanisms since an inoculum of  $10^2$  cultured cells killed 100% of immunosuppressed mice with an average survival time similar to that for mice inoculated with cells grown *in vivo*.

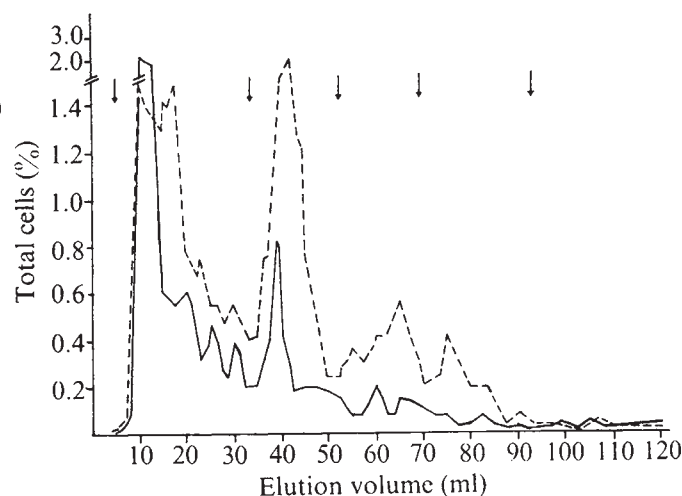
Cultured A-10 cells regained the tumorigenicity observed for cells grown *in vivo* when subsequently passaged as an ascitic tumour. Thus the tumorigenicity of this murine adenocarcinoma depended on conditions of tumour cell growth.

The elution profile of A-10 cells from con A-affinity columns also depended on conditions of cell growth (Fig. 1). Tumours grown *in vivo* contained a large subpopulation of cells eluted with PBS (not initially binding the column) and few cells were released by subsequent addition of sugar competitors for con A binding. In contrast, cultured A-10 tumour contained virtually no PBS-eluted subpopulation, and was characterised by subpopulations released with the different sugar solutions.

The elution profile of cultured A-10 cells was evaluated after the first and second passage in normal mice. As Fig. 2 shows, the first passage of cultured cells *in vivo* gave subpopulations characteristic of cells grown both *in vivo* and *in vitro*, that is, cells eluted with PBS, and large numbers of cells released by sugar solutions. The tumorigenicity of these cells was comparable with that observed for cultured

cells (Table 1). But, after the second passage of cultured cells *in vivo*, the elution profile had reverted almost completely to that of tumour grown *in vivo*. A large subpopulation was defined by elution with PBS, and few cells were released from the column by sugar. This profile correlated with a marked increase in tumorigenicity.

The antigenic properties of tumour cells and the expression of tumour-associated antigens have been altered by conditions of growth before<sup>8-11</sup>, but few studies have correlated surface properties of intact cells with either progressive tumour growth or tumour rejection. Studies on the tumorigenicity and strain-specificity of another murine mammary tumour, TA3 (refs 12-15), have shown the presence of a



**Fig. 2** The elution profile of cultured A-10 tumour after the first (---) and second (—) passage in B6AF<sub>1</sub> mice. The percentage of total cells is shown as a function of elution volume (see Fig. 1).

membrane glycoprotein, epiglycan, which correlated with loss of strain-specificity of TA3-Ha cells. Epiglycan was lost reversibly after these cells were cultured, and correlated with a return of strain-specific tumorigenicity. When TA3-Ha cells were grown *in vitro* other membrane changes occurred<sup>15</sup>, including a marked increase in the molar proportion of mannose to total hexosamine. In the light of our results, the relative increase of this con A-binding carbohydrate is particularly interesting.

We have demonstrated that tumour cell subpopulations of different immunogenicity could be isolated by a method based on their expression of cell-surface carbohydrates<sup>6,7</sup>. In addition, complement-dependent cytotoxicity of serum from tumour-immune animals was inhibited by  $\alpha$ -methyl-D-mannoside, a sugar used to elute highly immunogenic cells from con A columns. It seems reasonable that the relative

**Table 1** Tumorigenicity of A-10 cells in B6AF<sub>1</sub> mice after growth *in vivo* or *in vitro*

| Inoculum, growth conditions, host                                          | No. survivors/No. inoculated | Survival time |
|----------------------------------------------------------------------------|------------------------------|---------------|
| $10^2$ Cells, <i>in vivo</i> normal host                                   | 0/10                         | 23.2 ± 2.3    |
| $10^3$ Cells, <i>in vivo</i> normal host                                   | 0/10                         | 21.0 ± 1.8    |
| $10^2$ Cells, <i>in vitro</i> normal host                                  | 5/10                         | 27.1 ± 0.8    |
| $10^2$ Cells, <i>in vitro</i> immunosuppressed host                        | 0/10                         | 23.0 ± 0.6    |
| $10^2$ Cells, <i>in vitro</i> to <i>in vivo</i> , 1st passage, normal host | 4/5                          | 33.0          |
| $10^3$ Cells, <i>in vitro</i> to <i>in vivo</i> , 1st passage, normal host | 3/5                          | 31.0          |
| $10^2$ Cells, <i>in vitro</i> to <i>in vivo</i> , 2nd passage, normal host | 0/5                          | 24.2 ± 0.6    |
| $10^3$ Cells, <i>in vitro</i> to <i>in vivo</i> , 2nd passage, normal host | 0/5                          | 18.6 ± 1.7    |

Tumorigenicity of A-10 cells was determined by transplantability into B6AF<sub>1</sub> mice. Tumour cells grown *in vivo* were washed once in 1% ammonium oxalate (to lyse erythrocytes), followed by two washes in PBS. Cells grown *in vitro* were collected from logarithmic-phase cultures by a rubber policeman, followed by two washes in PBS. Cells for all experiments were > 90% viable, as judged by Trypan blue exclusion. Immunosuppression of B6AF<sub>1</sub> mice was by whole body irradiation, 450 rad, 24 h before injection of tumour. Intraperitoneal injections contained the appropriate number of cells in 0.2 ml of PBS. Survival time of mice represents the arithmetic mean ± s.e.



amount of mannose-like carbohydrates on the cell surface could influence the antigenic expression of tumour cells.

On the basis of our observations, together with the evidence of heterogeneity in tumour cell populations<sup>7,16</sup>, we can speculate on the tumorigenicity of A-10 adenocarcinoma. The subpopulation eluted with PBS comprised a substantial proportion of A-10 tumour grown *in vivo* (45%–50%) and was absent from cultured cells. But the presence of this subpopulation was not necessary for tumour growth *per se*, for cultured cells grew in immunosuppressed mice. This subpopulation may therefore be analogous to the phenotypic expression of epiglycan, and decreased expression of tumour-associated antigens; concomitant with a decreased proportion of mannose-like moieties at the cell surface.

The subpopulations of cells released by sugar, predominantly a feature of cultured cells, would be the inverse analogue of the above situation. The increased proportion of cell-surface con A-binding moieties could result in an increased expression of tumour-associated antigens and decreased tumorigenicity, except in the immunosuppressed host. We observed a 300-fold increase in the agglutinability of A-10 cells by con A after the cell line was established *in vitro*, which was consistent with increased expression of mannose-like moieties (J.J.K., unpublished results). These subpopulations would be selected against by passage of tumour *in vivo*, and the PBS-eluted phenotype would become dominant (Fig. 2). Tumorigenicity would be restored in the normal host.

Although more complex explanations are possible, the concept of tumour-cell subpopulations which depend on tumour-cell environment may have implications for the way in which tumour-cell properties ultimately influence the relationship between tumour and host.

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## Characterisation of Aleutian disease antigens

ALEUTIAN DISEASE (AD), which affects certain *Mustelidae*, is a persistent viral infection with development of non-neutralising antibody and gammopathy<sup>1–3</sup>. Some of the antigens developed in the course of AD are detectable by counter-immunoelectrophoresis (CEP) using sera from chronically infected mink. We have obtained evidence of two antigenic forms, Ag-1 and Ag-2, which have been characterised by molecular size, primary protein subunit components and by electron microscopy. Hollow ring-like structures, similar to those described with such viruses as  $\phi$ X174<sup>4</sup>, rat virus, Haden, adeno-associated virus and others<sup>5</sup>, were found associated with the AD virus (ADV) and seem to be the predominant antigenic component of Ag-1.

The CEP antigens, Ag-1 and Ag-2, were prepared from liver homogenates of ADV-infected mink by repeated Freon extraction and pelleting as described by Cho and Ingram<sup>6</sup>. These extracts were analysed by CEP at each step during the re-extraction procedure. Subsequently, samples rich in Ag-1 and Ag-2 were analysed by velocity sedimentation, molecular filtration and equilibrium density sedimentation. Further analyses consisted of electron microscopy and SDS-polyacrylamide gel electrophoresis of purified antigens.

Electrophoresis of antigens Ag-1 and Ag-2 by CEP revealed that they had different electrophoretic mobilities (Fig. 1). When Ag-1 and Ag-2 were mixed in varying proportions (threefold serial dilutions of Ag-2 and a constant amount of Ag-1), they consistently showed two precipitin bands when resolved by CEP against pooled AD antisera. CEP patterns revealed that Ag-2 was gradually lost during preparative steps consisting of repeated Freon extraction and pelleting; whereas Ag-1 persisted in these conditions. This formed the basis for the partial fractionation of Ag-2 and Ag-1 since Ag-2 was the predominant component in

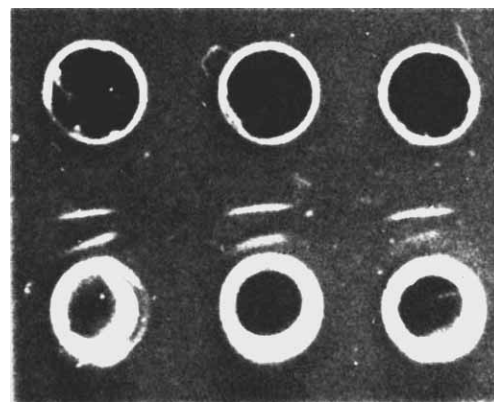


Fig. 1 Precipitation of Ag-1 and Ag-2 in CEP. Top wells (5 × 7.5 cm plate containing 1% agarose, 0.02 M barbital-acetate buffer, pH 8.2) contained mixtures of antigenic forms Ag-1 and Ag-2 with a constant amount of Ag-1 mixed with varying amounts of Ag-2 (threefold dilutions with concentrations decreasing from left to right). The bottom wells contained AD antisera. Ag-1 and Ag-2 were prepared from livers of pastel mink infected 8 d previously with 1 ml 10% w/v liver suspension, Connecticut strain ADV. The method of Cho and Ingram<sup>6</sup> was used which consisted of homogenisation, low speed centrifugation, and three repeated extractions of supernatants with Freon (Du Pont, trichlorotrifluoroethane). The antigens were pelleted from the aqueous phase by high speed centrifugation (180,000g for 90 min) to yield Ag-2. Further repeated Freon extraction (1 part Freon, 3 parts antigen solution) followed by high speed centrifugation yielded only Ag-1 activity. The Ag-1 was further purified by equilibrium density centrifugation (CsCl); fractions containing peak Ag-1 activity (1.33–1.35 g cm<sup>-3</sup>) were dialysed and used in the above precipitations.



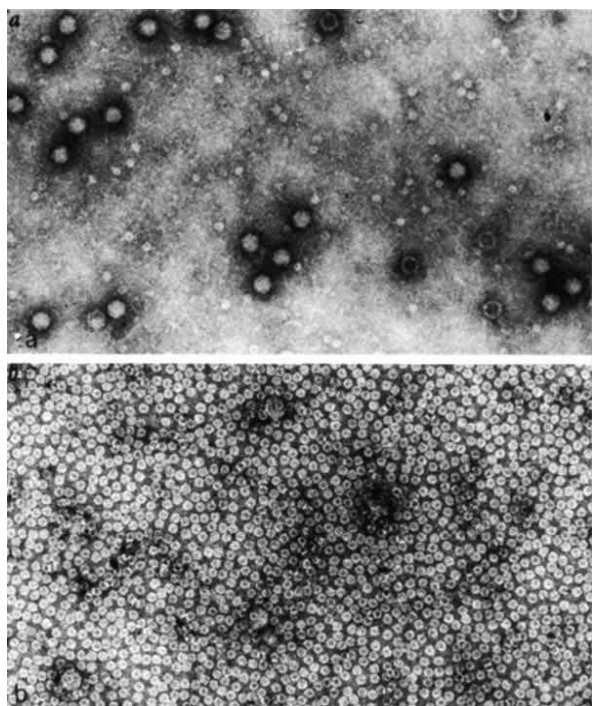


Fig. 2 *a* and *b*, Electron micrographs of Ag-1 component (pooled CsCl fraction 1.33–1.35). *a*, Icosahedral virus particles as well as ring-like structures (stained with sodium phosphotungstic acid, pH 7.0). *b*, Similar material stained with uranyl acetate, pH 4.2. Magnification: *a*, 100,000; *b*, 117,500.

intermediate (three times) Freon extracted preparations. The relative titre of Ag-2 in these preparations was about 100-fold greater than Ag-1 when estimated by endpoint titrations. On the other hand, after five extractions with Freon, Ag-1 was the only detectable antigenic structure which formed a precipitin band. Although the antigenic forms, Ag-1 and Ag-2, differed in mobility in CEP, they seemed to have similar molecular weights of  $2.5 \times 10^6$  (gel filtration through Biogel A-15m) and similar sedimentation coefficients (80S).

Ag-1 was further purified by density gradient centrifugation in CsCR as described in Fig. 1. Fractions containing peak antigenic activity ( $1.33\text{--}1.35 \text{ g cm}^{-3}$ ) were pooled and used for electron microscopy (Fig. 2) and for polyacrylamide gel electrophoretic analyses (Fig. 3).

Electron micrographs of the CsCl fractions containing the CEP antigen showed some intact particles of 27 nm and a large population of ring structures of 13 nm (Fig. 2). Cho and Ingram<sup>7</sup> have also described both ring structures and intact ADV particles. The intact particles cannot be the predominant CEP antigen, Ag-1, because of the low sedimentation coefficient (80S) of Ag-1. Since there were practically no empty particles in the preparation which otherwise could have accounted for this 80S antigen, it seems that the ring structures comprised the CEP antigen, Ag-1. We cannot say whether these structures are generated from intact or defective virus particles as a result of isolation procedures or whether they have accumulated as the result of defective assembly.

The subunit components of Ag-1 as determined by SDS-polyacrylamide gel analysis had molecular weights of 30,000, 25,000 and 15,000 (Fig. 2), which, if they are viral coded, are typical of a picornavirus polypeptide configuration<sup>8</sup> rather than a parvovirus polypeptide pattern, since parvoviruses usually have subunits of molecular weight  $>50,000$  (ref. 9). Since there is always a possibility of cleavage occurring in the primary protein subunits of Ag-1, by the procedures used here for iodination of Ag-1, classification of the Ag-1 as a derivative of a parvovirus or picornavirus

would have to be attempted by independent methods. Ag-1 purified from mink infected with the Canadian ADV isolate (F. J. Depauli Ontario Veterinary College, Guelph) showed a polypeptide composition of similar molecular weights. We are pursuing comparative structural and serological characterisation of the subunit proteins of Ag-1 from different strains of ADV. It is conceivable that different strains of ADV may be distinguished on the basis of their subunit compositions.

Ag-2 is a fragile structure which does not produce a discrete band in CsCl density gradients ( $1.2\text{--}1.5 \text{ g cm}^{-3}$ ) and has not yet been characterised by electron microscopy. The acrylamide gel analyses of Ag-2 (Fig. 2) showed the presence of only one protein subunit of molecular weight 15,000.

Antigenic activity of Ag-2 was not detectable after RNase treatment but was after DNase; whereas Ag-1 was resistant to both. Incubation of Ag-2 with  $10 \mu\text{g ml}^{-1}$  RNase at  $37^\circ\text{C}$  for 30 min completely destroyed the Ag-2 CEP antigenic activity. It has been suggested that the  $\delta$  peptide of picornavirus is associated with virion RNA. It is conceivable that Ag-2 represents such a viral substructure; however, it could also represent an association of viral protein with host RNA. The only antigenic form that could be isolated from

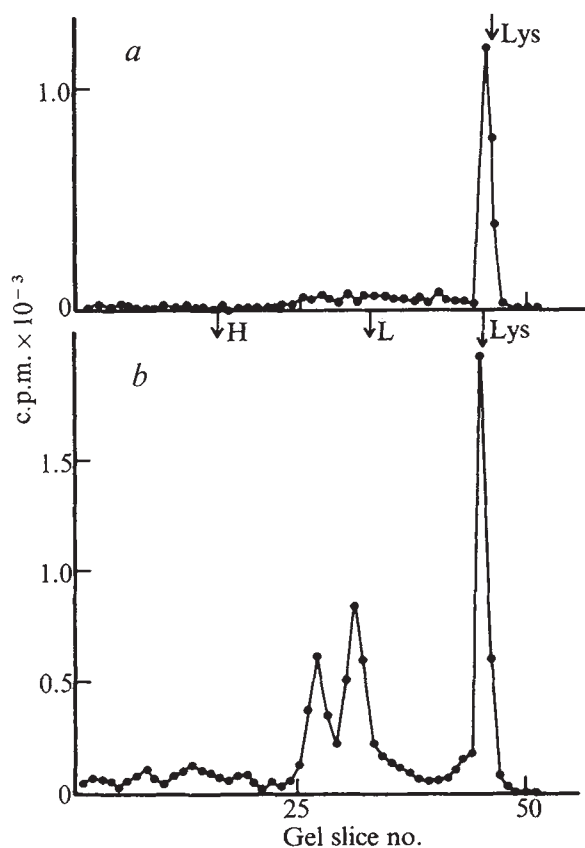


Fig. 3 Electrophoresis of dissociated  $^{125}\text{I}$ -labelled Ag-2(*a*) and Ag-1(*b*) on 10% SDS-polyacrylamide gels. Aliquots of Ag-1 and Ag-2 (shown in Fig. 1) were labelled with  $\text{Na}^{125}\text{I}$  using the method of Greenwood *et al.*<sup>10</sup> and dialysed against 0.02 M barbitol-acetate buffer, pH 8.2, and precipitated in CEP by antibody. The CEP plates were then extensively washed with PBS until free of unreacted components (established by autoradiographs). The precipitin bands were cut out, heated sufficiently in a water bath to melt the agarose and diluted in dissociation buffer (8 M urea-3% SDS at pH 8.0) before analysis on SDS-polyacrylamide gel electrophoresis. These precipitin bands were reduced and alkylated<sup>11</sup> in the dissociating buffer and dialysed for 12 h in Tris-HCl buffer, pH 6.8, before SDS-polyacrylamide gel electrophoresis<sup>12</sup>. The total gel was cut into 51 slices and counted in a Nuclear Chicago counter. Gels containing samples were compared with standards of human IgG heavy (H) and light (L) chains and lysozyme (Lys) run separately for molecular weight computation.

mink naturally infected in ranch conditions was Ag-2. Further studies on biological and immunological activities of Ag-2 would help in determining whether its accumulation has a role in the pathogenesis of chronic AD.

In these studies we have described properties of two CEP antigens; one is associated with ring structures and the other, which seems to be a nucleoprotein, is present in large amounts especially in mink with chronic AD. Protein subunits of these antigens can be obtained in sufficient purity for primary structural characterisations. Characterisation of these proteins would enable us to determine the relationship of AD virus to other viruses and to establish a basis for the unique biological properties<sup>2</sup> of different ADV strains.

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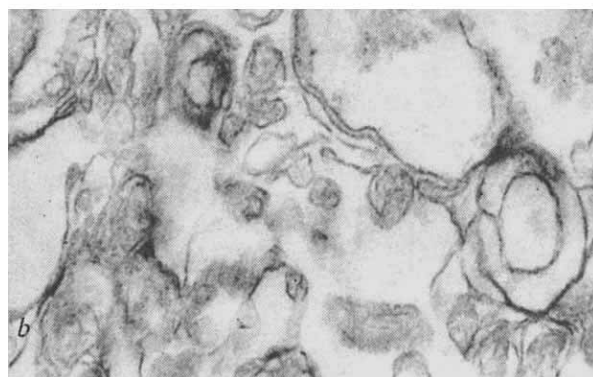
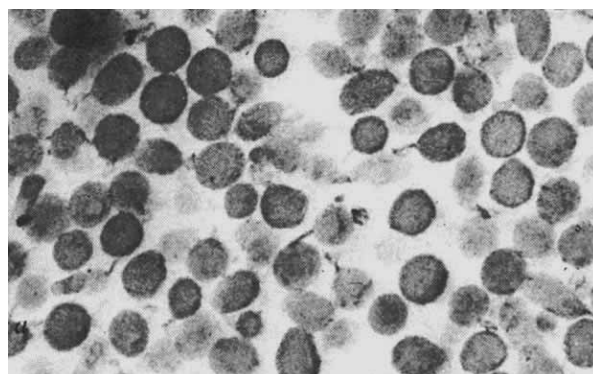
Reprint requests to Walker Laboratory SKI, Rye, N.Y. 10580.

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## Release of diacylglycerol-enriched vesicles from erythrocytes with increased intracellular $[Ca^{2+}]$

WHEN the intracellular  $Ca^{2+}$  concentration of human red cells was increased by treatment of the cells with A23187, a divalent cation ionophore, they changed shape from discocyte to echinocyte<sup>1-3</sup> and the 1,2-diacylglycerol content of their membranes was increased<sup>4-6</sup>. In these experiments it seemed that microvesicles budded off from the microvillus-like projections on the echinocytes<sup>2,3</sup>. We have now isolated the microvesicles and examined their composition and structure.

Packed washed human red cells (100 ml), isolated from blood approximately 1 week after collection into acid citrate-dextrose were incubated for 90 min at 37 °C with 10 ml of a medium containing A23187 ( $2 \mu g ml^{-1}$ ), 0.154 M NaCl, 2 mM  $CaCl_2$  and 10 mM HEPES-NaOH buffer,



**Fig. 1** Electron micrographs of intact microvesicles (a), and membranes isolated from glycol-lysed microvesicles from human erythrocytes treated with  $Ca^{2+}$  and ionophore A23187 (b). Samples were fixed with 4% glutaraldehyde followed by 1%  $OsO_4$ . Thin sections of material embedded in Epon were stained with uranyl acetate and lead citrate before examination in a Philips EM301 electron microscope. ( $\times 57,000$ ).

pH 7.4. Cells were sedimented and washed with 90 ml of the same medium. They were then stored at 2 °C for 16 h and were again sedimented and washed. During these procedures cell lysis was about 3%. The two supernatants were centrifuged separately at 20,000g for 30 min and each yielded a bright red pellet overlaid by a pink fluffy layer (probably the membranes from lysed cells). The fluffy layer was removed carefully by aspiration and discarded; the red pellets were retained. No additional material was sedimented by centrifugation at  $10^5 g$  for 60 min. Cells subjected to the same treatment in the absence of A23187 showed slightly more lysis, but centrifugation of their incubation medium yielded only traces of red pellet. Subsequent analysis showed no significant differences between the red pellets obtained from A23187-treated cells after 90 min and 16 h, and we concluded that these were successive collections of the same type of material and they could be pooled.

**Table 1** Lipid analysis of human erythrocytes and microvesicles derived from them

|                                      | Untreated cells (4)* | Cells treated with $Ca^{2+}$ and ionophore<br>Residual cells (4)* | Microvesicles (6)* |
|--------------------------------------|----------------------|-------------------------------------------------------------------|--------------------|
| Phospholipid                         | 56 ± 2†              | 56 ± 2                                                            | 54 ± 2             |
| Cholesterol                          | 44 ± 2               | 44 ± 2                                                            | 45 ± 2             |
| 1,2-Diacylglycerol                   | 0.05 ± 0.025         | 0.3 ± 0.05                                                        | 0.8 ± 0.05         |
| % Total phospholipid in subfractions | 100                  | 81 ± 5                                                            | 18 ± 3             |
| Phospholipid class                   |                      |                                                                   |                    |
| Sphingomyelin                        | 25.8 ± 1.7†          | 26.7 ± 1.9                                                        | 28.5 ± 1.7         |
| Phosphatidylcholine                  | 32.0 ± 1.6§          | 30.7 ± 0.8                                                        | 27.8 ± 1.4§        |
| Phosphatidylserine                   | 10.5 ± 0.9           | 10.5 ± 0.1                                                        | 12.0 ± 1.2         |
| Phosphatidylethanolamine             | 32.0 ± 1.6           | 31.6 ± 1.5                                                        | 32.7 ± 1.2         |

\*Figures in parentheses refer to the number of samples.

†Values are given as mol % of total lipids excluding glycolipids ± s.d.

‡Values are given as mol % of total phospholipids ± s.d.

§The difference between these values is statistically significant ( $0.05 > P > 0.02$ ).

Lipids were extracted and analysed as described previously<sup>4</sup>. Cholesterol was determined by the method of Brown *et al.*<sup>18</sup>.



Electron microscopy showed that the pellets consisted mainly of small spherical bodies about 100 nm in diameter with an internal electron density similar to that of erythrocytes (Fig. 1a); these are the characteristics expected of microvesicles budded off from the microvilli of A23187-treated cells<sup>3</sup>. When the pellet was frozen and thawed and then subjected to glycol-induced lysis<sup>3</sup>, more than 90% of its haemoglobin was released, and the remaining particulate material was sedimented, by centrifugation at 70,000g for 30 min, as a pale pink pellet consisting of membrane fragments, some of which seemed to be larger than the profiles of the original vesicles (Fig. 1b).

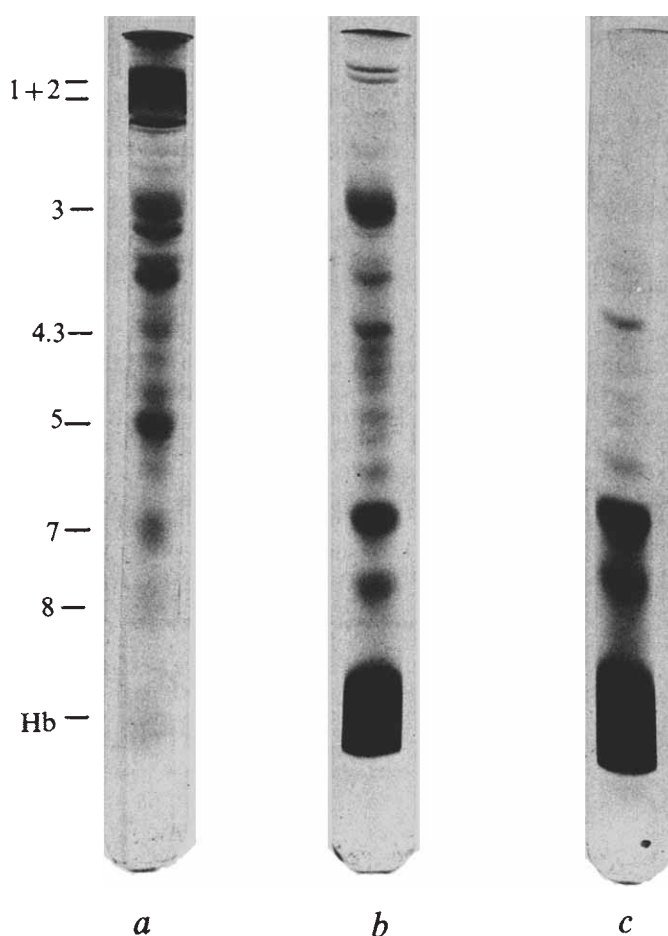
The ratio of total lipid (phospholipid plus cholesterol) to protein for the microvesicle membrane fraction was approximately 1.4 mg lipid per mg protein but, since haemoglobin accounted for about half of the remaining protein, the ratio

of lipid to non-haemoglobin protein was about 2.8. The equivalent value for haemoglobin-free erythrocyte ghosts is about 0.7 mg lipid per mg protein<sup>3</sup>; the microvesicle membranes were therefore markedly depleted in protein during their formation and separation from erythrocytes.

Lipid and polypeptide analyses were made on the original cells, on the A23187-treated cells remaining after the liberation of the microvesicles, and on the microvesicle fraction. The latter fraction contained 15–20% of the total cholesterol and phospholipid of the original cells, but less than 1% of their cytoplasm, as defined by haemoglobin content: this is the result expected from the much higher surface to volume ratio of vesicles compared with erythrocytes. As reported previously<sup>2–4</sup>, there was a marked increase in 1,2-diacylglycerol content in cells with an increased intracellular  $\text{Ca}^{2+}$  concentration. The change was even more pronounced in the microvesicles: this fraction contained only a fifth of the cell lipids but almost half of the newly formed diacylglycerol (Table 1). In addition, the microvesicle fraction was depleted in phosphatidylcholine by about 12% compared with the starting material, consistent with the suggestion that phosphatidylcholine is the substrate for  $\text{Ca}^{2+}$ -stimulated diacylglycerol production<sup>2–4</sup>.

As expected from its low protein to lipid ratio, the microvesicle fraction was deficient in polypeptide material compared with the normal membranes (Fig. 2a and b). This deficiency was due mainly to the almost complete loss of certain components which are prominent in normal membranes, particularly bands 1 and 2 (spectrin) and band 5 (actin)<sup>6,14</sup>. Periodate-Schiff staining of gels revealed that the major glycoprotein component of the erythrocyte (glycophorin) was also missing from the microvesicles. In contrast, band 3 was prominent both in normal membranes and in the microvesicles (Fig. 2a and b). Acetylcholinesterase, an enzyme which occupies a superficial site on the surface of normal red cells, was also retained in the microvesicles. Some polypeptides of the microvesicle fraction were cytoplasmic components which had passed into the budding microvesicles; comparison of Fig. 2b and Fig. 2c suggested that, in addition to haemoglobin, these may have included bands 7 and 8, and possibly band 4.3. In some experiments in which cells were isolated and treated with A23187 within 1 d of collection of the blood, the microvesicles were markedly depleted even of band 3. It is not known why the membrane protein complement of the microvesicles was sensitive to the period of storage of the blood before its exposure to ionophore.

The microvesiculation which occurs in response to increased intracellular  $\text{Ca}^{2+}$  seems likely to be caused by the increase in diacylglycerol content in the erythrocyte membrane, particularly since the microvesicles themselves have the highest content of this lipid. Outward vesiculation involves membrane fusion through interactions at the cytoplasmic surface of the microvillus-like projections formed initially. Diacylglycerol, which is a fusogenic lipid<sup>7,8</sup>, is probably produced in the inner surface of the membrane bilayer where it could mediate the fusion of cytoplasmic surfaces. Neither cell-cell fusion nor internal vesiculation, both of which involve fusion of outer membrane faces, was seen with ionophore treatment. This contrasts with the situation where diacylglycerol is formed in the outer lipid leaflet of erythrocyte membranes using exogenous phospholipase C, when inward vesiculation occurs<sup>9</sup>. These observations emphasise the asymmetrical nature of diacylglycerol-associated membrane budding processes. The asymmetry of the budding reaction probably arises because of the curvature induced by insertion of diacylglycerol into one leaflet of the lipid bilayer; the contraction in area of this leaflet may be a direct consequence of the formation of diacylglycerol<sup>10</sup>. The asymmetry is probably preserved only for as long as a gradient of the fusogenic molecule is maintained between the two lipid leaflets of the membrane. For diacyl-



**Fig. 2** Sodium dodecyl sulphate (SDS)-polyacrylamide gels of: *a*, human erythrocyte ghosts prepared by glycol lysis<sup>5</sup>; *b*, microvesicles released from human erythrocytes treated with  $\text{Ca}^{2+}$  and ionophore A23187; *c*, cytosol fraction prepared by centrifugation of osmotically lysed erythrocytes at 15,000g for 15 min. *a* and *b* contained 35 nmol of phospholipid and *c* contained about 300  $\mu\text{g}$  of haemoglobin. Samples were dissolved in 10% sucrose, 4% SDS, 1%  $\beta$ -mercaptoethanol, 10 mM phosphate buffer, pH 7.0, and heated at 95 °C for 1 min before application to 6% polyacrylamide gels. Electrophoresis was carried out at 4 mA per gel, using a buffer consisting of 100 mM sodium phosphate, pH 7.0, containing 0.1% SDS. Gels were stained with Coomassie blue.



glycerol it seems likely that equilibration between the two leaflets is fairly rapid<sup>9</sup>. Heating or prolonged incubation would assist equilibration between the two leaflets and this may explain why A23187-treated cells, which do not fuse immediately at 37 °C, can be made to fuse at 47 °C or on prolonged storage<sup>11</sup>.

The microvesicle membranes were markedly deficient in protein relative to the original erythrocyte membrane, suggesting that membrane fusion leading to outward vesiculation is more likely to occur in protein-depleted areas. This finding is consistent with a general model of membrane fusion proposed by Lucy *et al.*<sup>12</sup>. This model, however, envisaged the removal of all intrinsic proteins from the area of the membrane involved in fusion, whereas our data demonstrate that at least one major intrinsic protein (band 3)<sup>13</sup> is still present in the microvesicle membrane. Furthermore, the proteins most notably absent from microvesicles are spectrin and actin, extrinsic proteins which form a meshwork covering the inner surface of the membrane<sup>14</sup> where they might prevent fusion of lipid regions. It may be necessary for such extrinsic proteins to be removed from membranes before they become able to fuse.

Perhaps by virtue of their modified composition, particularly their decreased protein and increased diacylglycerol content, the microvesicle membranes may be prone to fuse with one another; this would contribute to the formation of extended membrane systems from microvesicles subjected to freeze-thawing, lysis and ultracentrifugation (Fig. 2b).

It has been suggested<sup>12,15</sup> that production of fusogenic lipids *in situ* might play a part in physiological membrane fusion events such as exocytosis or virus-induced cell fusion. The physiological role of diacylglycerol production at the inner surface of the erythrocyte membrane by an endogenous Ca<sup>2+</sup>-stimulated phospholipase C is not understood, but is clearly associated with echinocytosis and outward vesiculation. Such vesiculation, possibly as a result of declining or defective Ca<sup>2+</sup> homeostasis, could explain the loss of membrane components which occurs gradually in ageing cells<sup>16</sup> and more rapidly in hereditary spherocytic erythrocytes<sup>17</sup>.

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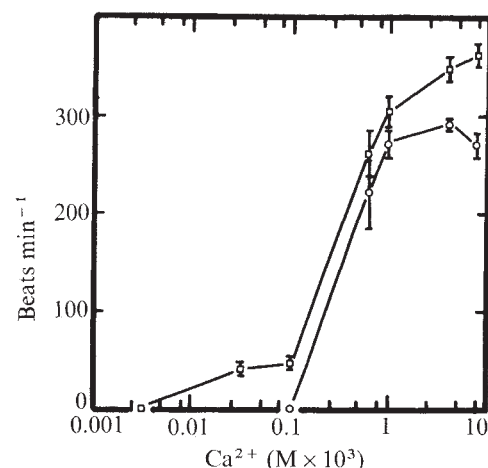
## Calcium ions regulate cyclic AMP and beating in cultured heart cells

ALTHOUGH the transport of Ca<sup>2+</sup> is known to be involved in the control of beating by cultured heart cells, little is known

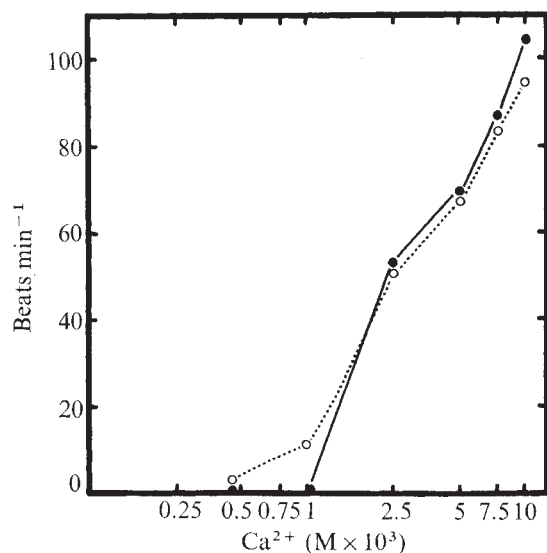
about the regulation of this process. There is evidence that catecholamines affect heart cells through their influence on cyclic AMP<sup>1-4</sup>. In this way they might affect Ca<sup>2+</sup> transport and, as a result, contractility. Our experiments with beating heart cells now suggest that both noradrenaline and Ca<sup>2+</sup> control the concentration of cyclic AMP in the cells which, in turn, regulates the utilisation of Ca<sup>2+</sup>. Addition of noradrenaline or depletion of Ca<sup>2+</sup> in the external medium results in cells which beat in a lower concentration of external Ca<sup>2+</sup> compared with controls.

Our experiments were carried out with rat heart cells prepared and cultured as before<sup>5-6</sup>. In a preliminary experiment noradrenaline (10<sup>-5</sup> M) increased the cellular concentration of cyclic AMP (as determined by the method of Gilman<sup>7</sup>) from 3 to 8 pmol per mg protein within 2 min. It seemed therefore that since cyclic AMP has been reported to increase Ca<sup>2+</sup> uptake, noradrenaline should also increase the utilisation of Ca<sup>2+</sup> by beating heart cells. To test this, the beating of heart cells was measured in the presence of different concentrations of Ca<sup>2+</sup>, decreasing from 8 × 10<sup>-3</sup> M, in the presence and absence of noradrenaline (Fig. 1). The rate of beating decreased with the decreasing concentration of Ca<sup>2+</sup>, and beating stopped at 10<sup>-4</sup> M Ca<sup>2+</sup>. In the presence of noradrenaline, however, beating was sustained down to 3 × 10<sup>-5</sup> M Ca<sup>2+</sup>. Thus, noradrenaline increased the ability of the heart cells to utilise Ca<sup>2+</sup> for beating.

We also studied the effect of Ca<sup>2+</sup> on the sensitivity of the cells to Ca<sup>2+</sup> and on the level of cyclic AMP. We measured the concentration of external Ca<sup>2+</sup> needed to reinitiate beating in heart cells partially depleted of Ca<sup>2+</sup> by incubation in low Ca<sup>2+</sup> medium for 2 h. (During this time, membrane-bound Ca<sup>2+</sup> and most of the easily exchangeable Ca<sup>2+</sup> is lost<sup>8,9</sup>.) Cells ceased beating at a concentration of Ca<sup>2+</sup> below 2.5 × 10<sup>-4</sup> M. In cultures preincubated in 3 × 10<sup>-6</sup> M Ca<sup>2+</sup> medium for 2 h, beating was restored by 4.5 × 10<sup>-5</sup> M Ca<sup>2+</sup>. This is six times less than the concentration of Ca<sup>2+</sup> needed to maintain the beating of cells preincubated in 'normal' medium, which contains 10<sup>-3</sup> M Ca<sup>2+</sup>. In another experiment cells incubated in 4.5 × 10<sup>-5</sup> M Ca<sup>2+</sup> stopped beating as expected, but within 2 h beating resumed spontaneously (Fig. 2). Thus the cells in low Ca<sup>2+</sup> developed a sensitivity to Ca<sup>2+</sup> and gained an ability to beat at the lower level. The requirement for external Ca<sup>2+</sup> was



**Fig. 1** Effect of noradrenaline (10<sup>-5</sup> M) and Ca<sup>2+</sup> on beating of heart cells. Rat heart cells were prepared as before<sup>5,6</sup> and incubated for 2 d in CMRL (1415AM) culture medium<sup>14</sup> supplemented with 5% foetal calf and 5% horse serum. Fresh medium was added 2 h before the experiment. Experimental medium was made with serum previously dialysed with Ca-free Tyrode solution; CaCl<sub>2</sub> was added to yield the indicated final Ca<sup>2+</sup> concentrations, taking into account the basal level of Ca<sup>2+</sup> measured by atomic absorption spectrophotometry. The rate of beating was determined visually after 20 min of incubation. The temperature was maintained at 37 °C. Data points represent the average of three or four plates; the vertical bars represent standard error of the mean. □, plus noradrenaline; ○, minus noradrenaline.

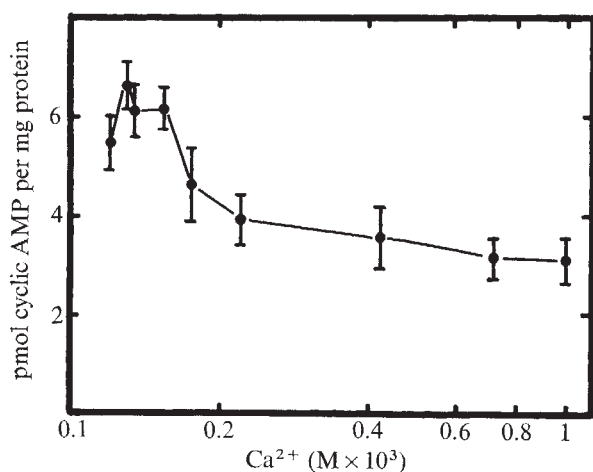


**Fig. 2** Beating of heart cells conditioned in high or low  $\text{Ca}^{2+}$  medium. Two-day-old cultures were incubated in fresh medium for 2 h before the addition of experimental medium (●). Beating rates in duplicate were measured 10 min after treatment. ○, Beating rates of heart cells treated as above but with an additional incubation in  $4.5 \times 10^{-5}$  M  $\text{Ca}^{2+}$  for 2 h before the addition of experimental medium. The solid symbol at  $4.5 \times 10^{-5}$  M  $\text{Ca}^{2+}$  indicates the beating rate 10 min after the cells were placed on this medium. The open symbol at  $4.5 \times 10^{-5}$  M  $\text{Ca}^{2+}$  indicates the beating rate 2 h after the cells were placed on this medium.

quantitatively dependent on previous exposure of the cells to  $\text{Ca}^{2+}$ . Cells incubated in a medium with high  $\text{Ca}^{2+}$  had a greater minimum external  $\text{Ca}^{2+}$  requirement to maintain beating than did cells incubated in a low  $\text{Ca}^{2+}$  medium. This indicated that the external  $\text{Ca}^{2+}$  concentration is involved in controlling the effect of  $\text{Ca}^{2+}$  on beating, and perhaps in controlling the intracellular level of  $\text{Ca}^{2+}$ .

If cyclic AMP influences  $\text{Ca}^{2+}$  transport, and in turn the external  $\text{Ca}^{2+}$  concentration also influences  $\text{Ca}^{2+}$  transport, it is possible that  $\text{Ca}^{2+}$  influences the concentration of cyclic AMP. To test this, we measured the direct effect of

**Fig. 3** Effect of external  $\text{Ca}^{2+}$  on cellular concentration of cyclic AMP. Two-day-old cultures were incubated for 2 h in growth medium with different  $\text{Ca}^{2+}$  concentrations, and cyclic AMP was determined. Each point represents an average of four plates. The vertical bars represent standard error of the mean. A  $t$  test was performed on grouped cyclic AMP values between  $10^{-4}$  M and  $2 \times 10^{-4}$  M and between  $2 \times 10^{-4}$  M and  $10^{-3}$  M. The level of significance was  $<0.001$ .



$\text{Ca}^{2+}$  on cyclic AMP. In a preliminary experiment  $10^{-5}$  M noradrenaline increased the concentration of cyclic AMP in cells incubated in  $10^{-3}$  M  $\text{Ca}^{2+}$  from 3 to 8 pmol per mg protein. Cells in low  $\text{Ca}^{2+}$  medium responded by an increase of cyclic AMP from 3 to 12 pmol per mg protein. After 1 h the cyclic AMP in cells incubated with noradrenaline in  $10^{-3}$  M  $\text{Ca}^{2+}$  had decreased to 5 pmol per mg protein, and with noradrenaline in low  $\text{Ca}^{2+}$  medium it had fallen only to 9 pmol per mg protein. Thus, in cells incubated in a medium containing noradrenaline and a low  $\text{Ca}^{2+}$  concentration, cyclic AMP reached a greater concentration for a longer time than in cells in medium containing more  $\text{Ca}^{2+}$ . Similar effects of noradrenaline on cyclic AMP have been reported for renal tubules in the presence of different concentrations of  $\text{Ca}^{2+}$  (ref. 10).

If cyclic AMP is related to the sensitivity of heart cells to  $\text{Ca}^{2+}$  then its concentration should respond directly to changes in external  $\text{Ca}^{2+}$ . When cells were incubated for 2 h in various concentrations of  $\text{Ca}^{2+}$ , their content of cyclic AMP increased from 3 to 6 pmol per mg protein as the concentration of  $\text{Ca}^{2+}$  decreased from  $10^{-3}$  M to  $10^{-4}$  M (Fig. 3). Thus a decrease in cellular  $\text{Ca}^{2+}$  increased the cyclic AMP and decreased the external  $\text{Ca}^{2+}$  requirement for beating, perhaps through the concomitant increase in cyclic AMP. In this way  $\text{Ca}^{2+}$  may regulate its own effect on contractility.

$\text{Ca}^{2+}$  may affect the concentration of cyclic AMP by an effect on cyclic AMP phosphodiesterase. This enzyme is stimulated by a modulator protein which is activated by  $\text{Ca}^{2+}$  (ref. 11). It has been suggested that  $\text{Ca}^{2+}$  is the limiting factor in the normal physiological state. Thus, a decrease in  $\text{Ca}^{2+}$  would cause a decrease in cyclic AMP phosphodiesterase activity and a resultant increase in cyclic AMP. Brooker<sup>12</sup> has reported that the level of cyclic AMP varies cyclically in relation to the beating cycle. This could be caused by the oscillation in the cytosol  $\text{Ca}^{2+}$  level which results from increased uptake of  $\text{Ca}^{2+}$  by the sarcotubular vesicles during the diastolic part of the beating cycle. It is also interesting that we have evidence that the  $\text{Ca}^{2+}$  protein activator of cyclic AMP phosphodiesterase is located on the sarcoplasmic reticulum<sup>13</sup>.  $\text{Ca}^{2+}$  has multiple roles in cardiac contractility, and we suggest that it has a role in regulating its own utilisation, perhaps through its control of cyclic AMP.

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## Alteration of electrical activity in molluscan neurones by cyclic nucleotides and peptide factors

ELECTRICAL activity in certain molluscan neurones is subject to long lasting synaptic and hormonal modulation<sup>1-3</sup>. Evidence from several systems suggests that cyclic nucleotides mediate alterations in membrane properties in nerve and muscle cells<sup>6-8</sup>. We report here that, in certain neurones from the marine snail *Aplysia californica* and the land snail *Helix pomatia*, an increase in the intracellular concentration of cyclic nucleotides is accompanied by long lasting changes in electrical activity, and that physiological agents known to modify the electrical rhythms of these nerve cells, increase adenosine 3',5'-cyclic monophosphate (cyclic AMP) in *Helix* and *Aplysia* nervous tissue.

Identified neurones R15 (ref. 9) in *Aplysia* abdominal ganglion and F-1 (ref. 5) in *Helix* circumoesophageal ganglia exhibit similar patterns of endogenous electrical activity, in which an oscillating membrane potential underlies alternating periods of action potential bursts and interburst hyperpolarisations<sup>10</sup>. We have found that the phosphodiesterase inhibitor isobutylmethylxanthine (IBMX), added to the bath at concentrations between  $10^{-5}$  M and  $3 \times 10^{-4}$  M, has profound effects on the bursting rhythm of these cells (Fig. 1a). After a lag of 20–45 min there was a marked increase in both the duration and amplitude of the interburst hyperpolarisation, as well as in the frequency and number of action potentials per burst. Simply shifting basal membrane potential (by intracellular injection of hyperpolarising or depolarising current) did not produce this effect. Similar concentrations of the alkaloid papaverine, another potent phosphodiesterase inhibitor, altered bursting rhythm in the same way as IBMX. These results suggested that an increase in concentrations of cyclic nucleotide might be involved in

**Table 1** Effect of phosphodiesterase inhibitors on cyclic AMP in *Aplysia* abdominal ganglion

| Treatment                        | Cyclic AMP (pmol per mg protein) |
|----------------------------------|----------------------------------|
| Control                          | 14 ± 2 (9)                       |
| Papaverine, $10^{-5}$ M          | 22 ± 2* (4)                      |
| Papaverine, $3 \times 10^{-4}$ M | 29 ± 4† (5)                      |
| IBMX, $10^{-5}$ M                | 38 ± 6† (4)                      |

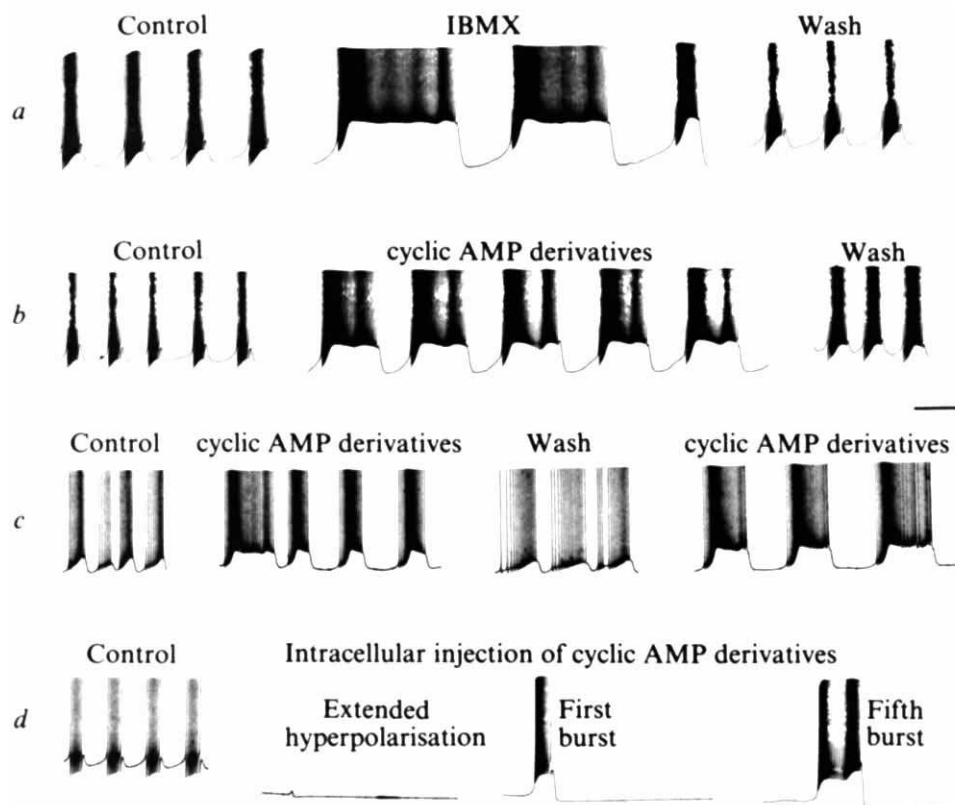
*Aplysia* abdominal ganglia were preincubated for 30 min at  $20 \pm 1^\circ\text{C}$  in control medium, then incubated for an additional 30 min in either control medium or medium containing the indicated concentration of inhibitor, before assay of cyclic AMP. After incubation, abdominal ganglia were frozen in liquid  $\text{N}_2$ , and homogenised immediately in acidified ethanol<sup>21</sup>. Homogenates were centrifuged to precipitate protein, and the ethanol supernatants were dried under reduced pressure. Cyclic AMP was measured by a binding assay<sup>22</sup>. Values are means ± s.e.m. for the number of ganglia shown in parentheses.

\*Significantly different from control,  $P < 0.05$ .

†Significantly different from control,  $P < 0.01$ .

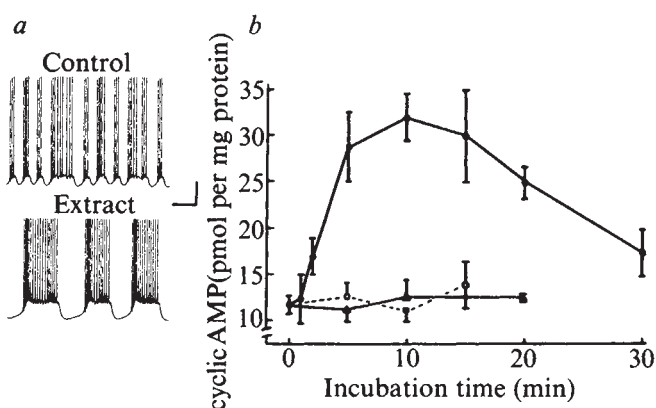
the change in electrical pattern; we found (Table 1) that cyclic AMP did accumulate in the *Aplysia* abdominal ganglion after treatment with IBMX or papaverine.

The action of IBMX and papaverine on bursting rhythm in neurones R15 and F-1 was not mimicked by externally perfused cyclic AMP or cyclic GMP in concentrations as high as  $2.5 \times 10^{-3}$  M. This might have been due to their failure to penetrate the cell membrane, or to their rapid destruction by intracellular phosphodiesterases before they reach the site of action. To circumvent the problem of membrane permeability, we injected solutions of cyclic AMP or cyclic GMP ( $2.5 \times 10^{-3}$  M in water, adjusted to pH 7.4) into R15 and F-1 through intracellular micropipettes<sup>21</sup>. Approximately 1 nl of solution was injected, and intracellular dilutions of 10–20-fold can be assumed. No change in bursting rhythm was observed in three preparations injected with cyclic AMP and two injected with cyclic GMP, indicating that low permeability is not sufficient to explain



**Fig. 1** Effects of IBMX and cyclic AMP derivatives on electrical activity in neurones R15 and F-1. Intracellular recording micropipettes were filled with either 3 M KCl or 0.6 M  $\text{K}_2\text{SO}_4$ . All experiments were performed at  $20 \pm 1^\circ\text{C}$ , between 0800 and 2100. *a*, Electrical activity was measured in cell R15 in an *Aplysia* abdominal ganglion during perfusion with normal *Aplysia* medium<sup>21</sup> (control), then 45 min after addition of *Aplysia* medium containing  $3 \times 10^{-4}$  M IBMX, and finally normal medium again (wash). *b*, Same as (*a*) except that a mixture of 8-benzylthio cyclic AMP and 8-(4-aminobutylamino) cyclic AMP ( $10^{-3}$  M each, obtained from ICN, Cleveland) was used in place of IBMX. *c*, Electrical activity measured in cell F-1 in *Helix* nervous system perfused with normal *Helix* medium (control), then *Helix* medium containing a mixture of cyclic AMP derivatives as in (*b*). After a 90-min wash with normal medium the derivatives were reintroduced. *d*, Effects of injection into R15 of the mixture of cyclic AMP derivatives ( $10^{-3}$  M each dissolved in water adjusted to pH 7.4). Within 6 min the cell hyperpolarised to a level 23 mV below that of the pre-injection interburst hyperpolarisation level, and remained silent for 30 min. When bursting returned,

the pattern was similar to that observed after application of phosphodiesterase inhibitors and cyclic AMP derivatives to the bath. Shown are the first and fifth (18 min after first) bursts after the extended hyperpolarisation. Calibration: 40 mV, 20 s.



**Fig. 2** Effects of *Helix* circumoesophageal ganglia extract on neuronal membrane properties and concentrations of cyclic AMP in *Helix* nervous system. Ganglia were removed from active<sup>25</sup> *Helix*, placed in 5-ml beakers containing 1 ml of *Helix* medium (modified from that described previously<sup>2</sup> by addition of amino acids and vitamins<sup>21</sup>), and preincubated at  $20 \pm 1^\circ\text{C}$  for 30 min before the start of the experiment. Acetic acid extract from 2.5 *Helix* circumoesophageal ganglia (total tissue wet weight 59 mg, total tissue protein content 1.6 mg), prepared as before<sup>2</sup> and lyophilised, was resuspended in 1 ml of *Helix* medium as test substance. Ganglia were incubated in medium either with or without extract, were frozen in liquid  $\text{N}_2$  and homogenised immediately in acidified ethanol. Homogenates were treated as for Table 1. Concentrations of cyclic AMP were measured by a binding assay<sup>21</sup>. The same resuspended extract was used for both (a) and (b). a, Membrane potential traces of cell F-1 before (top) or 20 min after (bottom) replacement of control bathing medium with extract-containing medium. The change in bursting rhythm induced by the extract could be reversed by prolonged washing in extract-free medium. Calibration: 20 mV, 10 s. b, Concentrations of cyclic AMP in circumoesophageal ganglia incubated in control medium (▲) or medium containing extract (●) for various times. Other circumoesophageal ganglia were incubated in 1 ml of medium, containing acetic acid extract of *Helix* foot muscle (total tissue wet weight 263 mg, total tissue protein content 9.4 mg), for various times (□). Data are mean  $\pm$  s.e.m. for 5-12 samples.

their lack of effect during application to the bath. Accordingly, we examined the effects of the 8-benzylthio, 8-parachlorophenylthio, and 8-(4-aminobutylamino) derivatives of cyclic AMP, all of which inhibit, and are highly resistant to breakdown by, phosphodiesterases<sup>12</sup>. These compounds are also potent activators of mammalian<sup>12</sup> protein kinases, and are more effective than cyclic AMP in altering electrical activity in cerebellar Purkinje cells<sup>13</sup>. All the derivatives, perfused at concentrations ranging from  $10^{-5}$  M to  $10^{-3}$  M, modified the bursting rhythm in cells R15 and F-1 after a 20-45-min lag (Fig. 1b and c). The effect was observed in all ten cells tested, and was transitory (although lasting at least 20 min) in six of them, even though the drug remained in the bathing medium. Reintroduction of the appropriate derivative, after a 1-h wash, caused a long-lasting (20 min or longer) reappearance of the modified bursting rhythm, again after a 20-45-min lag. Application of  $10^{-3}$  M  $N^6, O^2$ -dibutyl cyclic AMP to the bath had no effect, in agreement with a previous report<sup>14</sup>.

The 8-parachlorophenylthio derivative of cyclic GMP, perfused at  $10^{-3}$  M, did not affect the interburst hyperpolarisation in any of four R15 cells tested. Occasionally we noted an increased burst amplitude during treatment with this compound; this occurred preferentially after the long lasting, spontaneous i.p.s.p.s., already documented in R15 (ref. 15). In all four cells, treatment with 8-parachlorophenylthio cyclic GMP was followed by perfusion with the same concentration of 8-parachlorophenylthio cyclic AMP, either with or without an intervening wash. In all cases the cyclic AMP derivative potentiated the interburst hyperpolarisation, previously unaffected by the cyclic GMP derivative.

It is unlikely that the altered bursting rhythm reflects changes in neurones presynaptic to R15 and F-1. All three cyclic AMP derivatives which were effective when applied to the bath were also effective when injected into the cell bodies of R15 and F-1 (Fig. 1d). Again, a time lag was observed, suggesting that the lag after external application did not reflect a slow intracellular buildup of the derivatives, but rather was due to some slowly occurring metabolic process, or to diffusion of the derivatives to a site where they can act. Presumably, the membrane of the cell body, the site of action potential generation in bursting molluscan neurones<sup>16</sup>, is available to the compounds soon after their injection. In addition, tetrodotoxin-treated *Aplysia* ganglia, in which action potentials are suppressed, but slow oscillatory waves in R15 continue<sup>17</sup>, showed a lengthening of both hyperpolarising and depolarising phases of the cycle, when  $3 \times 10^{-4}$  M IBMX was added (our unpublished results). This indicates that the IBMX-induced change in R15 firing pattern (Fig. 1a) did not result from an alteration in firing pattern of some presynaptic neurone; it also demonstrates that the modified electrical rhythm did not require action potentials in R15.

Peptides from various sources can markedly alter membrane properties of certain molluscan neurones in a manner similar to that which we observed after exposure to cyclic nucleotides. Active factors include the vertebrate neurohypophysial hormones vasopressin and oxytocin<sup>1,18</sup>, as well as peptide-containing extracts from the nervous system of the terrestrial snail *Otala lactea*<sup>2</sup> and *A. californica*<sup>2,3</sup>. This suggests that cyclic AMP is an intermediary in the action of these agents, and we observed the accumulation of cyclic AMP in molluscan nervous system exposed to peptide hormones and peptide-containing extracts, at concentrations similar to those which alter neuronal membrane properties.

The effects of a *Helix* circumoesophageal ganglia extract on neuronal membrane properties and on cyclic nucleotide levels in *Helix* nervous system are shown in Fig. 2. As previously reported for cell 11 of *Otala*<sup>2</sup>, the extract markedly increased the depth and duration of the interburst hyperpolarisation, as well as the number and frequency of action potentials per burst, in cell F-1 of *Helix* (Fig. 2a), a result similar to that observed after addition of cyclic nucleotides to the medium bathing the nervous system. The same concentration of extract caused a large increase in cyclic AMP in *Helix* circumoesophageal ganglia (Fig. 2b). Concentrations of cyclic AMP were increased after as little as 2 min of incubation, reached a maximum at about 10 min, and

**Table 2** Effect of *Aplysia* bag-cell extract on cyclic AMP in *Aplysia* nervous system

| Tissue fraction           | Cyclic AMP (pmol per mg protein) |                  |
|---------------------------|----------------------------------|------------------|
|                           | Control                          | Bag-cell extract |
| Neurone R2 soma           | $23 \pm 1$ (9)                   | $20 \pm 3$ (8)   |
| Neurone R15 soma          | $16 \pm 6$ (9)                   | $19 \pm 6$ (9)   |
| Left upper quadrant of AG | $14 \pm 3$ (8)                   | $38 \pm 8^*$ (9) |
| Remainder of AG           | $12 \pm 1$ (8)                   | $30 \pm 5^*$ (8) |
| Connective nerves         | $15 \pm 3$ (5)                   | $13 \pm 4$ (5)   |

Acetic acid extracts of clusters of neurosecretory neurones (bag cells) from abdominal ganglia (AG) of *Aplysia* were prepared as described by Ifshin *et al.*<sup>2</sup>. The extracts were lyophilised and resuspended in appropriate volumes of *Aplysia* medium. AG were preincubated in *Aplysia* medium for 30 min at  $20 \pm 1^\circ\text{C}$ , then incubated in control medium or 1 ml of medium containing extract from four bag-cell clusters per ml, for 10 min before dissection. AG were then frozen in ethylene glycol-dry ice<sup>23</sup>, and identified neuronal cell bodies and segments of the AG were dissected and homogenised in acidified ethanol. Homogenates were treated as described for Table 1. R15 somata were assayed by radioimmunoassay<sup>24</sup>, other fractions by binding assay<sup>21</sup>. Values are means  $\pm$  s.e.m. for the number of samples shown in parentheses.

\*Significantly different from control,  $P < 0.01$ .



returned to near basal levels by 30 min. The factor in the extract responsible for the increase in cyclic AMP was stable at 100 °C or in 50% acetic acid, eluted in the exclusion volume of a Sephadex G-10 column and was destroyed by Pronase. In all these respects it is identical to the factor, tentatively identified as a peptide, which alters bursting rhythm in *Otala* cell 11 (ref. 2). An acetic acid extract of snail foot muscle affected neither bursting rhythm<sup>2</sup> nor concentrations of cyclic AMP (Fig. 2b).

The demonstration that an *Aplysia* bag-cell extract alters bursting rhythm in cell R15 of the abdominal ganglion<sup>3</sup>, and that cyclic nucleotides can mimic this effect, led us to investigate the influence of such an extract on cyclic AMP in different regions of the abdominal ganglion. No change in concentrations of cyclic AMP was observed in the cell bodies of the silent neurone R2 or the bursting neurone R15 after incubation of the abdominal ganglion with extract (Table 2). There was, however, a large increase in cyclic AMP in the left upper quadrant of the abdominal ganglion (which is rich in bursting neurones<sup>19</sup>), as well as in that portion of the ganglion remaining after removal of the R2 and R15 cell bodies and the left upper quadrant. The lack of any accumulation of cyclic AMP in the somata of R2 and R15 suggests that the response is confined to the neuropil, which contains both neuronal processes and glia<sup>20</sup>. Since connective nerves which are rich in stellate glial cells<sup>20</sup> did not accumulate cyclic AMP in the presence of bag-cell extract (Table 2), it is unlikely that this class of glia is involved.

Our findings are all consistent with the possibility that cyclic AMP mediates the effects of peptides on bursting rhythm, and may play a role in modulating the membrane properties of certain nerve cells.

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## N-demethylation of morphine in rat brain is localised in sites with high opiate receptor content

N-DEMETHYLATION of morphine, a principal biotransformation of this opiate alkaloid, has been demonstrated in several species including man<sup>1-4</sup>. The primary site of the reaction is the liver<sup>5,6</sup> but its existence in the central nervous system (CNS) has been inferred from experiments both *in vitro* with rat brain slices<sup>7</sup>, and *in vivo* with hepatectomised

rats<sup>8</sup>. The enzymatic N-demethylation of morphine and other biologically active tertiary amines has been studied extensively and both Axelrod<sup>5,6</sup> and Beckett *et al.*<sup>9</sup> have proposed that N-demethylation of narcotics is intimately involved in the mechanism of analgesia and the development of tolerance to and dependence on these agents. Subsequently, other investigators have questioned the validity of these hypotheses<sup>10,11</sup> and critical reviews of the experimental support of these theories have been published<sup>12,13</sup>. Belleau and Morgan<sup>14</sup> have revived interest in the biological significance of the N-demethylation of narcotics by proposing a provocative concept of clastic binding to opiate receptors in which the process of N-demethylation participates in conjunction with the opiate receptor in the mechanism of opiate action. This concept requires that the location of the sites of N-demethylation within the CNS is proximal to or coincident with that of the opiate receptors. Previous reports<sup>7,8,15</sup> have described the N-demethylation of morphine by the whole brain, and our study was initiated to locate the reaction at specific sites. To do this *in vivo*, we used a new, highly sensitive assay. A mixture of 6-<sup>3</sup>H-morphine<sup>16</sup>, and N-<sup>14</sup>CH<sub>3</sub>-morphine (Amersham/Searle), of known isotope ratio, was injected into each rat and, after killing, the isotope ratio of selected tissues was determined accurately by combustion in a tissue oxidiser. N-demethylation of this mixture generates <sup>14</sup>C-containing fragments originating from the N-methyl group, but the N-demethylated products of the reaction contain only <sup>3</sup>H. The former are dissipated from the site of reaction much more rapidly than the latter, so that an increase in the ratio of <sup>3</sup>H to <sup>14</sup>C in a specific tissue would serve to indicate the presence, and also the extent, of any *in situ* N-demethylation.

The results of a series of experiments are listed in Table 1. The liver is a known site of N-demethylation<sup>5,6</sup> and in every study this tissue contains a ratio of <sup>3</sup>H to <sup>14</sup>C significantly greater than that of the blood and can therefore be considered as a positive control. The only other tissues showing a similar increase in the isotopic ratio were in the CNS and consisted of the hypothalamus, the medial thalamus and the corpus striatum. The ratio of <sup>3</sup>H to <sup>14</sup>C in these tissues was significantly greater than that in the blood, indicating that the peripherally administered morphine was N-demethylated at these sites. In contrast, none of the other tissues examined had a ratio of <sup>3</sup>H to <sup>14</sup>C greater than that of the blood, suggesting that they were not sites of N-demethylation. This evidence, however, need not be exclusive since further dissection of the other brain areas could provide fraction(s) with disproportionate isotope ratios which were obscured by the isotope contents of the larger section in our work.

These results were obtained in experiment 1 in which a pharmacological dose of morphine (~10 mg kg<sup>-1</sup>) was injected. In experiments 2 and 3, in which tracer or sub-pharmacological doses of morphine were injected, there were no comparable changes in isotope ratio, in spite of continued clear and undiminished evidence of the N-demethylation in the liver. This failure to observe isotope ratio differences in the central tissues in these experiments cannot be due to insensitivity of detection for although the tissues contained less material, the amount of radioactivity present in the CNS regions was comparable with that in experiment 1. This suggests that N-demethylation in the brain proceeds only when pharmacological concentrations of the substrate are present, which tends to confirm the biological significance of the reaction. Alternatively, it is possible that morphine is demethylated in the CNS when low doses are present, but that the smaller amounts of N-demethylated products formed are lost from the site of the reaction within 30 min, and the loss of both isotopes results in failure to observe changes in ratio. In experiment 4, in which a pharmacological dose of morphine was used but the animals were killed after 60 min, there is significantly

**Table 1** Rat tissue isotope ratios after subcutaneous administration of 6-<sup>3</sup>H-morphine/*N*-<sup>14</sup>C-morphine (normalised to dose ratio = 1)

| Experiment      | 1                               | 2                               | 3           | 4                               | 5                               |
|-----------------|---------------------------------|---------------------------------|-------------|---------------------------------|---------------------------------|
| Blood           | 0.89 ± 0.02                     | 0.92 ± 0.05                     | 1.06 ± 0.04 | 0.92 ± 0.02                     | 1.02 ± 0.07                     |
| Cerebral cortex | 0.82 ± 0.08                     | 0.73 ± 0.08                     | 0.84 ± 0.09 | 0.89 ± 0.04                     | 0.85 ± 0.11                     |
| Hypothalamus    | 1.04 ± 0.07<br><i>P</i> < 0.001 | 0.83 ± 0.12                     | 0.96 ± 0.10 | 1.00 ± 0.07<br><i>P</i> < 0.02  | 1.22 ± 0.13<br><i>P</i> < 0.01  |
| Medial thalamus | 1.24 ± 0.25<br><i>P</i> < 0.001 | 0.79 ± 0.19                     | 0.92 ± 0.13 | 0.98 ± 0.07<br>NS               | 1.40 ± 0.23<br><i>P</i> < 0.01  |
| Corpus striatum | 1.15 ± 0.08<br><i>P</i> < 0.001 | 0.84 ± 0.10                     | 0.95 ± 0.16 | 0.99 ± 0.06<br><i>P</i> < 0.02  | 1.20 ± 0.07<br><i>P</i> < 0.001 |
| Midbrain        | 0.83 ± 0.04                     | 0.69 ± 0.07                     | 0.87 ± 0.04 | 0.91 ± 0.07                     | 0.86 ± 0.10                     |
| Cerebellum      | 0.82 ± 0.06                     | 0.77 ± 0.03                     | 0.92 ± 0.12 | 0.88 ± 0.04                     | 0.93 ± 0.08                     |
| Medulla         | 0.88 ± 0.04                     | 0.82 ± 0.08                     | 0.95 ± 0.10 | 0.97 ± 0.07                     | 0.97 ± 0.11                     |
| Liver           | 1.20 ± 0.07<br><i>P</i> < 0.001 | 1.23 ± 0.04<br><i>P</i> < 0.001 | 1.45 ± 0.16 | 1.25 ± 0.09<br><i>P</i> < 0.001 | 1.21 ± 0.05<br><i>P</i> < 0.001 |

The recorded isotope ratios are the average for six male Sprague-Dawley rats (~250 g) used in each study. Significance (to blood) was calculated by Student's *t* test<sup>24</sup>. Animals were killed by cervical fracture, and the CNS was dissected as before<sup>25</sup>. Tissues were combusted in an Oxymat tissue oxidiser (Intertechnique). The tritiated water and <sup>14</sup>CO<sub>2</sub> released by each sample was trapped and counted in a Packard scintillation counter. In experiments 1, 2, 3 and 5, animals were killed 30 min after injection; in experiment 4, after 60 min. Doses were: experiment 1, 2.5 mg morphine per rat; 2, 0.2 mg morphine per rat; 3, 0.02 mg morphine per rat; 4, 2.5 mg morphine per rat; 5, 2.5 mg morphine + 0.1 mg naloxone per rat.

less evidence for *N*-demethylation in the hypothalamus, corpus striatum and medial thalamus, presumably due to the greater loss of the tritium-containing *N*-demethylated products from the site of the reaction during the longer time. The isotope ratios in the liver were not affected by the increase in time, suggesting either a continuing reaction or a greater retention time of the *N*-demethylated products in this organ.

The observed isotope ratio changes in specific central tissues could be interpreted to arise not from *in situ N*-demethylation of the 6-<sup>3</sup>H-morphine-<sup>14</sup>CH<sub>3</sub>-morphine mixture, but as a product of preferential uptake of *N*-demethylated compounds originating from the liver. Several lines of evidence argue against this interpretation. Demethylated (nor)-morphine and presumably other *N*-demethylated products cross the blood-brain barrier less effectively than the corresponding *N*-methyl derivatives<sup>17,18</sup> and this would lead to isotope ratio changes, which would be the reverse of those observed. Furthermore, in unpublished studies in which liver *N*-demethylation was abolished, changes were observed in the isotope ratio in the CNS. Finally, in experiment 4, in spite of continuing liver *N*-demethylation, the changes in isotope ratio in the CNS were reduced substantially with time.

Our work demonstrates that the *N*-demethylation of morphine in the brain of the rat is limited to specific sites which include the hypothalamus, medial thalamus and corpus striatum. These tissues have been reported to contain relatively high concentrations of opiate receptors<sup>19-22</sup>, whereas sites described as deficient in opiate receptors, such as the cerebral cortex, cerebellum and the medulla, showed no evidence of *N*-demethylation. Therefore there is coincidence between the sites of this biotransformation and the presence of opiate receptors, which supports the hypothesis that the receptor-mediated mechanism of opiate action involves *N*-demethylation. Further support for this concept was sought in experiment 5, in which the presence of the narcotic antagonist naloxone was expected to affect the demethylation of morphine in the CNS. But neither in the CNS nor in the liver were there any clear changes in morphine *N*-demethylation. These negative results suggest that either the reaction is not pharmacologically significant, or that the antagonist interferes with the chain of events subsequent to *N*-demethylation of the agonist. Alternatively, the antagonist may affect either the time scale or quantitative aspects of *N*-demethylation in a manner which does not become evident in the conditions we used.

We have previously utilised a double isotope procedure with *N*-<sup>14</sup>CH<sub>3</sub>-morphine and <sup>3</sup>H-naloxone in an effort to demonstrate displacement of the agonist by the antagonist in specific central sites<sup>23</sup>. We knew that *N*-demethylation might

distort the results and performed a reverse isotope experiment in which 6-<sup>3</sup>H-morphine and <sup>14</sup>C-naloxone were used. There was no evidence of any change in isotope ratio, and we assumed that *N*-demethylation of morphine does not proceed to a sufficient degree in the CNS to be detectable by these methods. These experiments, however, were carried out with sub-pharmacological doses of morphine. In subsequent experiments using pharmacological doses of *N*-<sup>14</sup>CH<sub>3</sub>-morphine and <sup>3</sup>H-naloxone we observed greater ratios of <sup>3</sup>H to <sup>14</sup>C in the medial thalamus and hypothalamus and interpreted these changes as indicative of displacement of morphine by the antagonist in these tissues. In view of these results, these interpretations must be revised since they were due to *N*-demethylation of the morphine and loss of the <sup>14</sup>C fragment from the tissue. The extent of this biotransformation is comparable with the 'displacement' observed. Displacement of agonist by antagonist from receptors *in vivo* is still presumed to occur, but in amounts not detectable by our procedures.

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## Immunocytochemical evidence for the presence of arginine vasotocin in the rat pineal gland

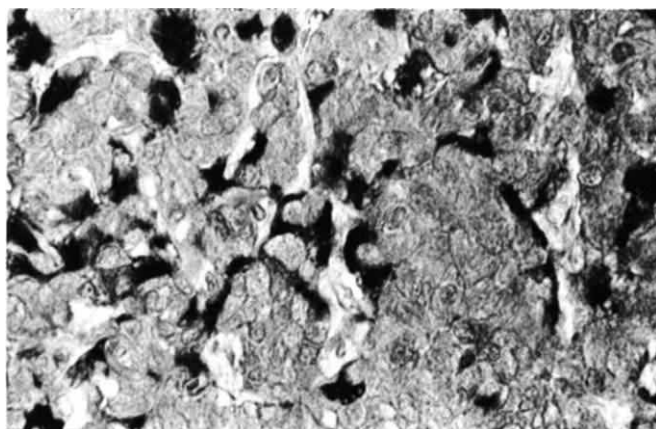
THE discovery that the pineal gland contained melatonin<sup>1</sup> provided the stimulus for a multidisciplinary approach to the study of this organ. Considerable evidence suggests that this gland affects reproductive physiology. For example, it contains biologically active polypeptides. One of the first of these to be identified was arginine vasotocin (AVT), initially isolated from extracts of bovine pineal gland<sup>2</sup>, and later identified chemically and found to have antigonadotropic properties<sup>3,4</sup>. There is physiological and radioimmunoassay evidence for the origin of AVT in the pineal gland. We now present immunocytochemical evidence for the presence of this polypeptide in the pineal gland.

AVT was conjugated to bovine serum albumin, and antiserum to the complex was prepared in adult male guinea pigs. The antiserum was absorbed with 1 mg of bovine serum albumin per ml of undiluted serum before use. Pineal glands and hypothalami from ten adult male rats were fixed in Bouin's solution and embedded in paraffin. Immunocytochemical staining was carried out with undiluted antiserum to AVT according to the procedure of Nakane and Pierce<sup>5</sup>. Controls similar to those described by Petrusz<sup>6</sup> were carried out to assure specificity of staining.

Because of the marked structural similarity of AVT, arginine vasopressin (AVP) and oxytocin, and because other investigators have claimed that the pineal gland contains AVP<sup>6</sup> and luteinising hormone releasing hormone (LHRH)<sup>9</sup>, establishment of the specificity of the antiserum to AVT was important. The findings summarised in Table 1 strongly suggest that the antiserum to AVT was specific for AVT. Moreover, the supraoptic nucleus, the paraventricular nucleus and the median eminence (regions reputedly rich in AVP, oxytocin, and LHRH, respectively) did not stain with antiserum to AVT, substantiating the immunocytochemical specificity of the antiserum to AVT.

Antiserum to AVT immunocytochemically stained a distinct population of cells in the rat pineal gland (Fig. 1). These cells were distributed diffusely throughout the gland. They were very irregular in form with extensive perivascular and intercellular processes. Cytoplasmic processes were frequently cupped around neighbouring cells. Most parenchymal cells were regular in outline and were identified as cells classically described as pinealocytes or chief cells. Their characteristic pale nuclei with infolded nuclear membranes

**Fig. 1** Light micrograph of a section from a rat pineal gland immunochemically stained with antiserum to AVT. The darkly staining reaction product of the Nakane-Pierce technique defines the presumptive AVT cells.



**Table 1** Immunological specificity of antiserum to AVT

|              | Anti-AVT | Anti-AVT<br>+<br>AVT | Anti-AVT<br>+<br>AVP | Anti-AVT<br>+<br>OT | Anti-AVT<br>+<br>LHRH |
|--------------|----------|----------------------|----------------------|---------------------|-----------------------|
| Pineal gland | +        | —                    | +                    | +                   | +                     |

Immunological specificity was tested by absorbing undiluted antiserum to AVT with 0.2 mg of AVT, AVP, OT, or LHRH, before immunochemical staining of rat pineal sections.

and many nucleoli were in marked contrast to the intensely staining, elongate nuclei of the immunochemically stained cells. The pronounced staining of the nuclei of the presumptive AVT cells was interesting because this was not normally observed after immunocytochemical staining of cells containing polypeptides.

These findings add further evidence for the presence of AVT in the pineal gland. Whether the immunocytochemically stained cells of this study represent a second line of pinealocytes or are glial in origin, and whether they synthesise or store AVT has yet to be determined.

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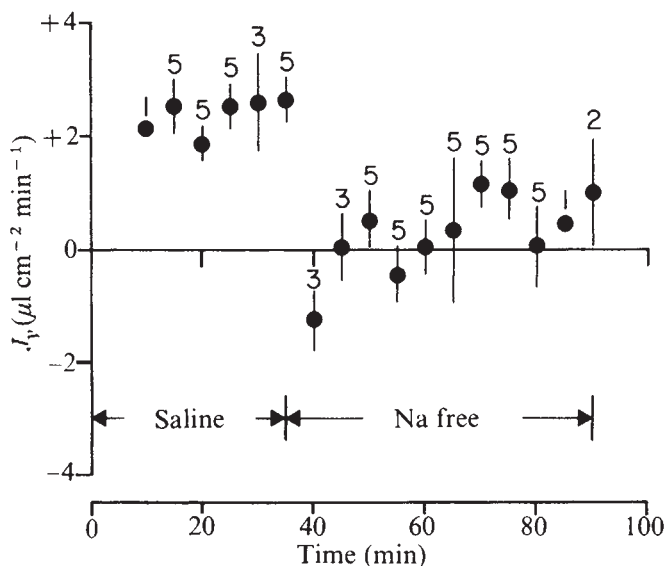
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## Functional roles of luminal sodium and potassium in water transport across desert scorpion ileum

IN terrestrial insects the rectum is a major site of fluid absorption and is, therefore, important in the physiological control of water balance. Water absorption in the insect rectum occurs against osmotic gradients greater than 300 mosmol l<sup>-1</sup> and apparently in the absence of net ion movements from lumen to haemolymph<sup>1,2</sup>. Evidence suggests that net water flow from the rectal lumen to haemolymph results from increases in intercellular space osmotic pressure due to the transport across lateral cell membranes of recyclable intracellular solutes<sup>3,4</sup>. Little is known about the mechanism of water transport in the insect gut anterior to the rectum nor about gastrointestinal processes involved in water regulation in other terrestrial arthropods. Scorpions are ideal organisms for measurements of arthropod ileal transport because this part of the gut extends nearly the entire length of the tail and can be isolated for *in vitro* perfusion. We present here preliminary information on a sodium-dependent, potassium-inhibited water transport mechanism in the ileum of the desert scorpion *Hadrurus arizonensis*.

Scorpions were anaesthetised lightly with chloroform, and the entire ileum (2 cm) was removed and placed in a saline medium. The ionic composition and osmotic pressure of the medium were based on data obtained from flame photometry, chloride titration and osmometry of haemolymph samples, as well as on values reported from other scorpion species in the literature<sup>5–7</sup>. This scorpion saline had an osmotic pressure



**Fig. 1** Effect of sodium-free perfusate on net water flux across scorpion ileum. Ileae were first exposed to normal saline on the luminal surface for 35 min and were subsequently perfused for 55 min with medium lacking sodium. Normal saline was present in the serosal bath at all times. A positive net water flux indicates movement from mucosa to serosa. Circles represent mean flux values, vertical lines are  $\pm 1$  s.e.m. and numbers signify sample size.

of 550 mosmol  $l^{-1}$ , a pH of 7.4, and consisted of the following ion concentrations in mM: Na, 281.5; K, 8.0; Ca, 10.0; Mg, 20.0; Cl, 260.0;  $HCO_3$ , 1.5;  $SO_4$ , 44. When incubation media of altered ionic composition were used, choline chloride was substituted for NaCl and KCl, and  $KHCO_3$  for  $NaHCO_3$ ; KCl concentrations were increased at the expense of those of NaCl ( $Na_2SO_4$  replacing the altered NaCl), and raffinose was added to maintain approximately equal osmotic pressures on both sides of the epithelium. Ionic concentrations of normal ileal fluid were also measured by flame photometry after collection and centrifugation in haematocrit capillary tubes.

Each end of the isolated ileum was mounted on epoxy-coated 18-gauge stainless steel needles with surgical thread, immersed in 3 ml of scorpion medium in a lucite chamber, and perfused with saline by means of a polystaltic pump at a flow rate of about  $125 \mu l \min^{-1}$ . Net water transport ( $J_v$ ) was measured by the procedures developed for perfused kidney nephrons<sup>8</sup> where the change in perfusate  $^{131}I$ -albumen concentration (Squibb), after passage through the tubular preparation, provides information about the extent and direction of water flow. The perfusion rate ( $V_o$ ,  $\mu l \min^{-1}$ ) was calculated as

$$V_o = \frac{^{131}I_{tot}}{[^{131}I_o]t}$$

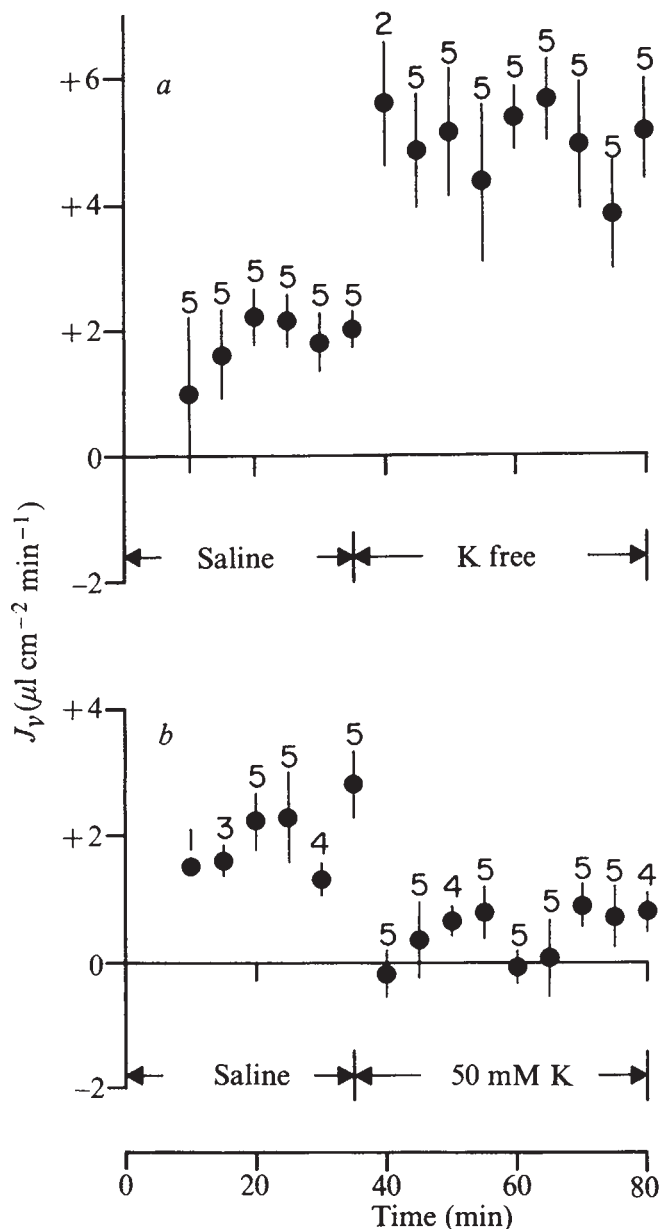
where  $[^{131}I_o]$  is the specific activity in the perfusion fluid (d.p.m.  $\mu l^{-1}$ );  $^{131}I_{tot}$  the total amount of radioactivity in a sample of ileal fluid collected after passage through the gut (d.p.m.), and  $t$  the duration of the collection period (min). The rate of collection ( $V_f$ ,  $\mu l \min^{-1}$ ) was estimated directly from the volume, determined gravimetrically to the nearest 0.1 mg, and duration of each collection. The net water transport rate  $J_v$  ( $\mu l \text{ cm}^{-2} \min^{-1}$ ) was calculated as

$$J_v = (V_o - V_f)/A$$

where  $A$  represents the estimated mucosal area ( $\text{cm}^2$ ) determined by considering the ileum as a cylinder. A positive value of  $J_v$  reflects net movement of water from mucosa to serosa. Ileae were typically perfused with  $^{131}I$ -albumen-labelled medium for the first 35 min (control interval), followed by perfusion for 45–55 min with medium of altered ionic composition containing the radioactive marker. Collections were made every 5 min. The serosal bath was periodically sampled for radioactivity

to test for leaks.  $^{131}I$ -albumen samples were counted in a Beckman LS-230 liquid scintillation spectrometer using a Triton X-100/toluene/Omnifluor (New England Nuclear) scintillation cocktail.

Figure 1 shows that when the transmural osmotic gradient was zero (determined by measuring directly the osmotic pressures of mucosal and serosal solutions),  $J_v$  in scorpion medium was  $+2.40 \pm 0.18 \mu l \text{ cm}^{-2} \min^{-1}$  (mean  $\pm$  s.e.m.;  $n = 24$ ). That this net movement of water was energy dependent was shown in another experiment where the saline bathing both ileal surfaces contained 1 mM NaCN and 1 mM iodoacetate. Net flow in these conditions was reduced to  $+0.14 \pm$



**Fig. 2 a**, Effect of potassium-free perfusate on net water flux across scorpion ileum. Ileae were first exposed to normal saline on the luminal surface for 35 min and were subsequently perfused for 45 min with medium lacking potassium. Normal saline was present in the serosal bath at all times. A positive net flux indicates water movement from mucosa to serosa. Symbols are as described in Fig. 1. **b**, Effect of increasing the potassium concentration of the perfusate from 8.0 mM (normal condition) to 50 mM on net water flux across scorpion ileum. Ileae were first exposed to normal saline on the luminal surface for 35 min and were subsequently perfused for 45 min with medium containing 50 mM potassium and normal sodium. Normal saline was present in the serosal bath at all times. A positive net flux indicates water movement from mucosa to serosa. Symbols are as described in Fig. 1.



**Table 1** Effects of starvation and dehydration of *Hadrurus arizonensis* on sodium and potassium concentrations of centrifuged ileal contents

| Ions in ileal fluid | Freshly fed*   | Starved and partially dehydrated† | Starved and severely dehydrated‡ |
|---------------------|----------------|-----------------------------------|----------------------------------|
| Na                  | 123.3±17.8 (5) | 177.0±38.6 (3)                    | 468.0±112.4 (7)                  |
| K                   | 274.9±41.5 (5) | 131.8±58.1 (3)                    | 83.2± 24.3 (7)                   |

Ion concentrations are expressed in mM (mean±s.e.m.) and numbers in parentheses represent sample sizes.

\*Scorpions fed crickets the day before sampling.

†Scorpions maintained without food at 25 °C and at about 30% relative humidity for 2 weeks before sampling.

‡Scorpions maintained without food at 35 °C and about 30% relative humidity for 4 weeks before sampling.

0.24  $\mu\text{L cm}^{-2} \text{ min}^{-1}$  ( $n=36$ ), which is not significantly different from zero ( $P>0.05$ ). Figure 1 also indicates that when ilea were perfused with Na-free medium (normal medium on serosal side), the net flux of water from mucosa to serosa was eliminated ( $J_v = +0.28 \pm 0.22 \mu\text{L cm}^{-2} \text{ min}^{-1}$ ;  $n=44$ ;  $0.20 > P > 0.10$ ). An analysis of variance test comparing  $J_v$  for the control group and net water flux for the Na-free preparations showed the two groups to be significantly different ( $P<0.005$ ).

When potassium ion was eliminated from the saline perfusate and sodium ion remained normal (281.5 mM) net water flux from the mucosal to serosal side of the preparation increased significantly ( $P<0.005$ ) above the control value of  $+1.79 \pm 0.26$  ( $n=30$ ) to  $4.92 \pm 0.29$  ( $n=42$ )  $\mu\text{L cm}^{-2} \text{ min}^{-1}$  (Fig. 2, top). An increase in luminal potassium concentration from 8.0 to 50.0 mM at constant luminal sodium concentration (281.5 mM) resulted in a significant decrease ( $P<0.005$ ) in mucosal to serosal  $J_v$  from a control value of  $+1.95 \pm 0.23$  ( $n=23$ ) to  $+0.43 \pm 0.14$  ( $n=43$ )  $\mu\text{L cm}^{-2} \text{ min}^{-1}$  (Fig. 2, bottom). The latter value is still significantly greater than zero ( $P<0.01$ ). Control  $J_v$  values from the above three experiments, compared using analysis of variance, were not significantly different ( $P>0.05$ ).

Scorpions that had eaten the day before sampling illustrated ileal sodium and potassium concentrations of  $123.3 \pm 17.9$  mM ( $n=5$ ) and  $274.9 \pm 41.5$  mM ( $n=5$ ), respectively (Table 1). Starving and dehydrating animals at temperatures up to 35 °C and for periods up to 4 weeks significantly increased the ileal sodium concentration ( $n=15$ ;  $P<0.05$ ) and significantly decreased the potassium concentration ( $n=15$ ;  $P<0.01$ ). In addition, the ratio of sodium to potassium was reversed. In freshly fed scorpions, Na/K was 0.46, whereas in starved and dehydrated scorpions Na/K was 5.6 (an ionic ratio that produced a net flux of water from lumen to blood in Fig. 2, bottom).

This is the first detailed study of a water transport mechanism in the ileum of a terrestrial arthropod, although Wigglesworth and Ramsay have suggested<sup>9,10</sup> that some water absorption occurs in this portion of the insect gut. Our data suggest that the ileal absorption of water in the desert scorpion occurs through a sodium-dependent, potassium-inhibited transport process. This mechanism is clearly adaptive as dehydration and starvation lead to intraluminal sodium and potassium concentrations which favour the uptake of water. In freshly fed animals excessive hydration could be reduced by the inhibitory interaction of potassium ion on the sodium-dependent water movement. Water balance in these organisms may depend on the relative sodium/potassium ratio present in the ileum. This will require further study.

The anatomical location of the inhibitory effect of potassium on sodium-dependent water movement remains unclear. The scorpion ileum, in contrast to that of insects, is unchitinised and has a tall columnar epithelium<sup>11</sup>. Therefore, a standing osmotic gradient<sup>12-14</sup> in the long intercellular spaces created by an electrogenic, energy-requiring lateral membrane cation pump could satisfactorily generate the transmural water fluxes observed. The rate of sodium efflux by the cation pump would be a function of the intracellular sodium concentration, which,

in turn, would depend on the luminal concentration of this ion. Potassium ion, derived from the ileal lumen could compete with sodium for attachment to this lateral membrane cation pump and convert its electrogenicity into a simple K-K exchange involving no net ionic fluxes. A reduction or elimination of net transmural water flow would then result from a decrease in the intercellular standing osmotic gradient. In contrast, competition between sodium and potassium for a shared carrier process in the apical membrane, followed by active sodium efflux across the lateral membrane could equally well account for the effects of ions on water flow described here. Further studies of ion fluxes in the scorpion ileum are clearly needed to establish more thoroughly their role in transmural water movements.

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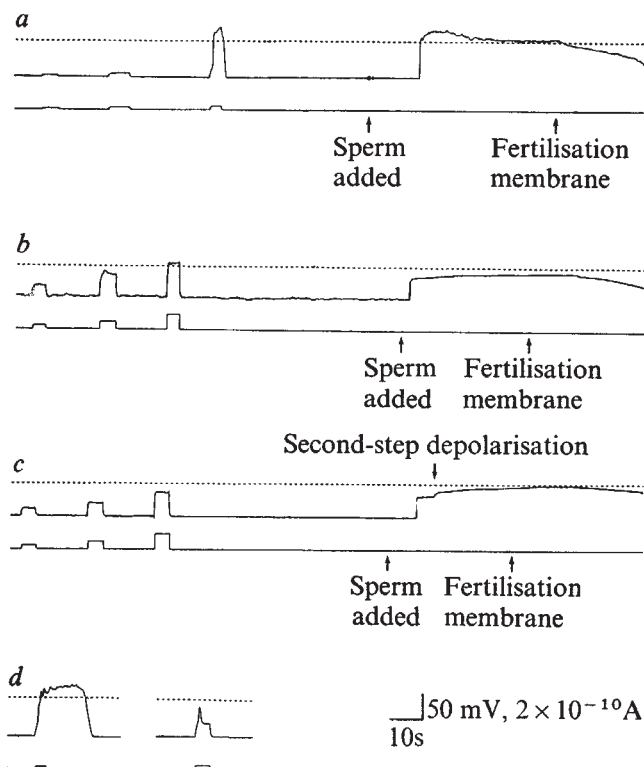
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## Fast block to polyspermy in sea urchin eggs is electrically mediated

THE prevention of polyspermy in sea urchin eggs is accomplished in two steps: a "fast" block, occurring within a few seconds of the entry of the fertilising sperm, and a "slow" block, associated with the cortical reaction (compare refs 1-4 with 5 and 6). Results presented here confirm the existence of the fast block and demonstrate that it is caused by the electrical depolarisation of the egg plasma membrane that accompanies the entrance of the fertilising sperm.

Eggs of *Strongylocentrotus purpuratus* were hand centrifuged and agitated mechanically with a jet of seawater, to remove their jelly coats. Some of the dejellied eggs adhered to the bottom of Falcon plastic Petri dishes, such that a microelectrode could be inserted. All experiments involved eggs with a stable resting potential of greater than -60 mV (Table 1). These values of resting potential are close to that calculated from independent ion tracer flux studies done on these eggs (my work in preparation with K. R. Robinson). A single microelectrode was used for both recording voltage and passing current, by means of a conventional bridge circuit. Experiments were done at 15 °C, in natural or artificial seawater, Tris-buffered at pH 8. In all experiments, the concentration was about  $10^6$  sperm per ml, which is within the range that can produce polyspermy in some batches of eggs, but not in most. Polyspermy was determined by observation of first cleavage: monospermic eggs divide into two equal cells, whereas polyspermic eggs



**Fig. 1** Action potentials and activation potentials in *S. purpuratus*. The top trace is voltage against time; the bottom trace is current against time. The dotted line indicates 0 mV. Before addition of sperm, each egg membrane was tested for electrical excitability by applying a series of small depolarising current pulses. *a*, Egg with regenerative response to current pulse; monospermic activation potential. *b*, Egg with non-regenerative response; monospermic activation potential. *c*, Egg with non-regenerative response; polyspermic activation potential. *d*, Other examples of the regenerative response; a strong response outlasting the stimulus, a weak response.

cleave abnormally, dispermic eggs usually producing three or four cells at once<sup>7,8</sup>.

Figure 1 shows intracellular electrical records from three eggs before, during and after addition of sperm. The fast depolarisation after the addition of sperm has been called the "activation potential"<sup>9-15</sup>. Within 3–30 s after the introduction of sperm near the egg, the egg membrane depolarises to a plateau at  $-30$ – $+20$  mV, and then after about 1 min begins to repolarise.

An interesting correlation was found between the plateau level of the activation potential (measured 3 s after its rise) and the occurrence of polyspermy (Table 1). Eggs with activation potentials which reached a plateau more positive than 0 mV were never polyspermic (none out of eight cases) and those which reached a plateau less positive than  $-10$  mV were sometimes polyspermic (seven out of 13 cases). These results suggest that the entry of extra sperm is prevented by the more positive-going activation potential.

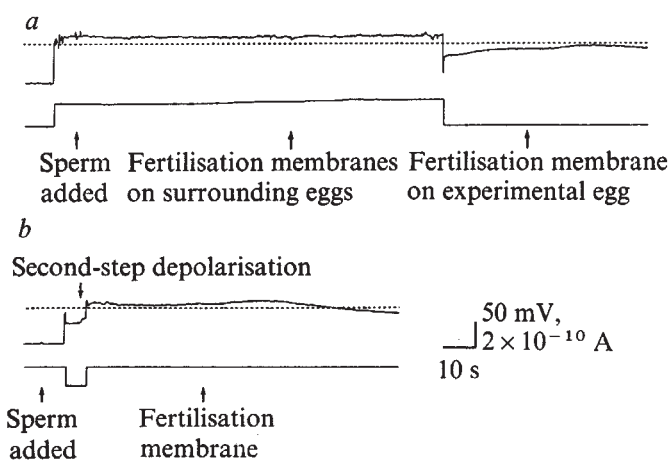
Figure 1c shows an activation potential in a polyspermic egg. About 5 s after the initial rise, there was a distinct second step depolarisation (see arrow). Eleven polyspermic activation potentials were observed (seven listed in Table 1, four not shown). All 11 showed this second step, and sometimes a third step. Ten monospermic activation potentials, as in Fig. 1b, were observed; of these, eight definitely did not show a second step. Two cases did show a second step; their nature is obscure. (In cases like that in Fig. 1a, with an oscillating plateau level, the steps could not be counted.) These results indicate that the second step depolarisation is associated with the entrance of a second sperm. The voltage at which this second step occurs is always more

negative than  $-10$  mV, suggesting that when the egg membrane is depolarised beyond  $-10$  mV, sperm penetration is less probable.

Figure 2a shows that when current was applied to hold the unfertilised egg membrane at a potential more positive than  $+5$  mV, sperm did not fertilise the egg. In spite of the presence of many active sperm adhering to the egg surface, no fertilisation membrane formed, and no electrical event resembling an activation potential occurred. In some experiments, the potential was held for more than 5 min, with repeated additions of sperm; but still no fertilisation occurred in the voltage-clamped eggs. (Surrounding eggs formed fertilisation membranes within 1 min of the first addition of sperm.) As soon as the current was turned off, fertilisation occurred: the egg depolarised, showed a typical activation potential, formed an apparently normal fertilisation membrane with the usual time course, and, if the activation potential was of the one-step form, first cleavage was normal, indicating that only one sperm had entered the egg. The same result was obtained with holding potentials ranging from  $+5$  to  $+130$  mV. In other experiments, the potential was held for about 10 min, without addition of fresh sperm, after which most sperm had become less active and incapable of fertilising eggs. When the current was turned off, the egg membrane returned to its original resting potential and showed the electrical properties characteristic of the unfertilised state. Morphologically, there was no sign of activation, and no development occurred. If more sperm were added, fertilisation and development proceeded in the usual way.

Fertilisation is prevented at  $+5$  mV; but it occurs at  $-10$  mV, as determined by the experiments reported in Table 2. The egg was depolarised enough to block fertilisation, and sperm were added. After 3–5 min, the voltage was lowered to a point where fertilisation occurred. Each experiment sets an upper and a lower limit on the threshold voltage for suppressing sperm entry. This is between  $+5$  and  $-10$  mV; at intermediate potentials, entry of sperm may depend on their concentration and activity, and on individual variation among eggs. This threshold is consistent with the following observations on activation potentials.

(1) Eggs whose activation potential plateau (measured 3 s after the initial rise) was more positive than 0 mV, are



**Fig. 2** Demonstration of potential dependence of sperm entrance. The top trace is voltage against time; the bottom trace is current against time. The dotted line indicates 0 mV. *a*, Suppression of fertilisation by positive holding potential. *b*, Induction of polyspermy by negative holding potential. In (*a*) the amount of current required to maintain the holding potential increased about 10% during the first 1–2 min after the current was turned on. This increase was seen even if sperm were not added. In some experiments, sperm were not added until this initial adaptation of the egg membrane was complete. The addition of sperm did not cause any change in the amount of current required to maintain the potential.



Table 1 Activation potentials for monospermic and polyspermic eggs

| Egg | Resting potential (mV) | Regenerative response | Activation potential plateau (mV) | First cleavage | No. of steps in rise of activation potential | Voltage at second step (mV) |
|-----|------------------------|-----------------------|-----------------------------------|----------------|----------------------------------------------|-----------------------------|
| A   | -75                    | Strong                | +23                               | M              | —                                            | —                           |
| B   | -69                    | Strong                | +17                               | M              | —                                            | —                           |
| C   | -68                    | Strong                | +15                               | M              | —                                            | —                           |
| D   | -76                    | Strong                | +14                               | M              | —                                            | —                           |
| E   | -77                    | Strong                | +12                               | M              | —                                            | —                           |
| F   | -74                    | Strong                | +10                               | M              | —                                            | —                           |
| G   | -71                    | Strong                | +6                                | M              | —                                            | —                           |
| H   | -71                    | Weak                  | 0                                 | M              | —                                            | —                           |
| I   | -71                    | Weak                  | -12                               | P              | 2                                            | -9                          |
| J   | -74                    | NT                    | -13                               | M              | 1                                            | —                           |
| K   | -68                    | Weak                  | -14                               | M              | 1                                            | —                           |
| L   | -75                    | Weak                  | -15                               | M              | 1                                            | —                           |
| M   | -56                    | NT                    | -17                               | P              | 2                                            | -15                         |
| N   | -60                    | None                  | -19                               | M              | 1                                            | —                           |
| O   | -63                    | None                  | -20                               | M              | 1                                            | —                           |
| P   | -79                    | Weak                  | -20                               | P              | 2                                            | -14                         |
| Q   | -71                    | Weak                  | -22                               | P              | 2                                            | -22                         |
| R   | -64                    | None                  | -23                               | P              | 2                                            | -22                         |
| S   | -70                    | Weak                  | -26                               | M              | 2                                            | -27                         |
| T   | -65                    | None                  | -29                               | P              | 2                                            | -26                         |
| U   | -78                    | NT                    | -35                               | P              | 3                                            | -35, -24                    |

The activation potential plateau was measured 3 s after the initial rise. Eggs were classified as monospermic (M) if first cleavage produced two equal cells; otherwise, they were classified as polyspermic (P). The regenerative response of the unfertilised egg was classified as strong if in response to a 5-s current pulse of  $3 \times 10^{-11}$  A, the membrane maintained a positive potential for the duration of the pulse. None means that the response was completely, or almost completely, non-regenerative. Weak means that there was some regenerative response, but it was not strong. NT, Not tested.

always monospermic. (2) The entrance of the second sperm in polyspermic eggs occurred at a voltage more negative than  $-10$  mV.

Application of current to bring the plateau of the activation potential to a more negative level facilitated entry of additional sperm (Fig. 2b). After the activation potential began, current was applied to hold the potential at  $-30$  mV. A second-step depolarisation occurred, then the current was turned off. Observation of first cleavage showed that this egg was polyspermic, whereas 98% of the surrounding eggs in the dish were monospermic. This experiment was repeated seven times, with holding potentials of  $-30 \pm 5$  mV. In all cases, experimental eggs were polyspermic and surrounding eggs were monospermic.

These experiments demonstrate that the electrical depolarisation occurring at fertilisation constitutes a fast block to polyspermy. It was frequently observed that when the electrically monitored egg was polyspermic, other eggs in the dish were also polyspermic (except in cases where current was applied to make an egg polyspermic). Therefore, the lack of a fast block to polyspermy is a property of a batch of eggs. Loss of the capacity to block polyspermy might be a symptom of egg deterioration, either in the adult, or after removal from the adult.

The rise of the activation potential can occur within 3 s of insemination. Part of this time is necessary for the sperm to swim to the egg surface and to attach to the vitelline membrane. Only then does the sperm fuse with the egg plasma membrane. Therefore, it can be concluded that the fast block to polyspermy is established in less than 3 s after fusion of the fertilising sperm. Quite possibly, the rise of the activation potential is a direct consequence of the disturbance of the egg membrane by sperm fusion. If this is the case, it follows from the rate of rise of the activation potential that the fast block to polyspermy is established in less than 1 s after fusion. (The rise from resting level to  $-10$  mV takes between 0.1 and 1 s.) The membrane remains in the depolarised state for about 1 min, after which the cortical reaction has occurred, thus providing a permanent block to polyspermy.

In each example in Fig. 1, a series of small depolarising current pulses was applied to the egg before sperm were

added. In the case shown in Fig. 1a, the stimulus pulse induced a regenerative response of the egg membrane. This response showed a threshold at about  $-50$  mV, and sometimes outlasted the stimulus (Fig. 1d); it has been observed in other echinoderm oocytes and eggs<sup>13-17</sup> and has been studied in sea urchin eggs by K. Takahashi (personal communication). It is an action potential of the sort seen in nerve and muscle (as distinct from the activation potential). Figure 1b and c illustrates eggs which did not show a regenerative response to depolarisation. These eggs showed less positive-going activation potentials after sperm addition, compared with the egg in Fig. 1a. This relationship between the presence of the regenerative response and the height of the activation potential is also shown in Table 1. The correlation indicated that the activation potential is caused partly by a regenerative response of the egg membrane. The data in Table 1 suggest that the sperm fusion depolarises the egg to a level of about  $-30$

Table 2 Voltage threshold for suppression of fertilisation

| Egg | Suppressing potential (mV) | Non-suppressing potential (mV) |
|-----|----------------------------|--------------------------------|
| A   | +6                         | -6                             |
| B   | +3                         | -11                            |
| C   | +2                         | -10                            |
| D   | +1                         | -10                            |
| E   | +5                         | -7                             |
| F   | +3                         | -12                            |
| G   | +5                         | -6                             |
| H   | +6                         | -5                             |
| I   | —                          | -2                             |

The egg membrane was held at the suppressing potential and sperm were added. About 1 min later, all the surrounding eggs had formed fertilisation membranes. 3-5 min after addition of sperm, the potential was reduced to the non-suppressing level: within 1-2 min, the experimental egg formed a fertilisation membrane. After removal of the electrode, balance of the WPI bridge circuit was tested by applying a current pulse of the same magnitude used in the experiment. If the imbalance was greater than 2 mV, data were not used. Error associated with the single microelectrode technique was small, because of the small currents used ( $0.3-3 \times 10^{-10}$  A), and the high input resistances of the cells (200-2,000 M $\Omega$ , depending on the degree of regenerativeness of the egg's response), compared with the resistances of the microelectrodes (40-80 M $\Omega$ ).

to  $-20$  mV. Then, in eggs which have the capacity to produce a regenerative response, the sperm-initiated depolarisation is amplified, bringing the egg membrane potential to a level which excludes additional sperm.

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## Translation of RNA from unfertilised sea urchin eggs does not require methylation and is inhibited by 7-methylguanosine-5'-monophosphate

At fertilisation, the rate of protein synthesis in the sea urchin embryo becomes several times greater than that observed in unfertilised eggs<sup>1–4</sup>. This increase does not require the synthesis of messenger RNA (mRNA), since it occurs both in the presence of actinomycin D (ref. 5) and in enucleated parthenogenetically activated egg fragments<sup>6</sup>. These observations led Spirin<sup>7</sup> to postulate the existence of preformed “maternal” mRNA in the eggs. Recent experiments have directly demonstrated the presence of mRNA in sea urchin egg cytoplasm by isolating it from postribosomal cytoplasmic fraction and translating it in a heterologous cell-free system<sup>8</sup>.

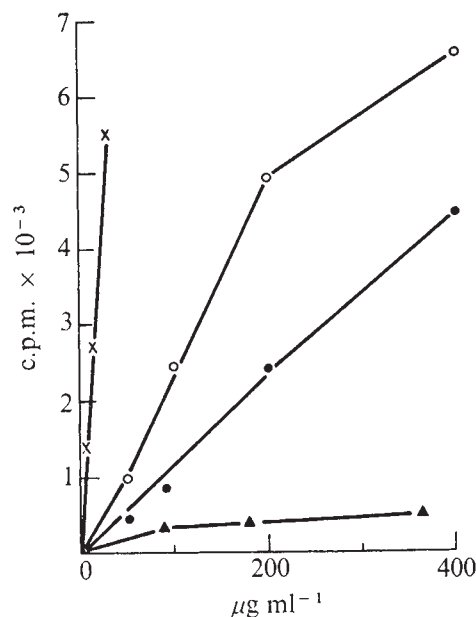
The mechanism(s) responsible for the increased rate of protein synthesis at fertilisation is still not understood. Some investigators have attributed this increased rate to the removal of an inhibitor of protein synthesis from the egg ribosomes<sup>9,10</sup>. Clegg and Denny<sup>11</sup>, however, have reported that ribosomes isolated from unfertilised eggs translate rabbit globin mRNA as efficiently as ribosomes isolated from zygotes when supplied with ascites cell postribosomal supernatant and reticulocyte initiation factors. These authors concluded that the low protein synthetic activity in unfertilised eggs is not attributable to the presence of inhibited ribosomes<sup>11</sup>.

Activation of mRNA by adenylation may also be important in the increased rate of protein synthesis<sup>12,13</sup>. The mRNAs coding for histones are never adenylated<sup>14</sup>, however, but make up a considerable proportion of maternal mRNA<sup>8,15</sup>. Adenylation thus does not have any role in the translational activation of this class of mRNAs, although it may be involved in increasing the stability of other mRNAs<sup>16</sup>. In this paper we discuss two other mechanisms for the activation of maternal mRNA which involve the methylation of the 5'-terminal nucleotide or the addition of 5'-terminal 7-methylguanosine.

The 5' termini of many viral and cellular mRNAs have been

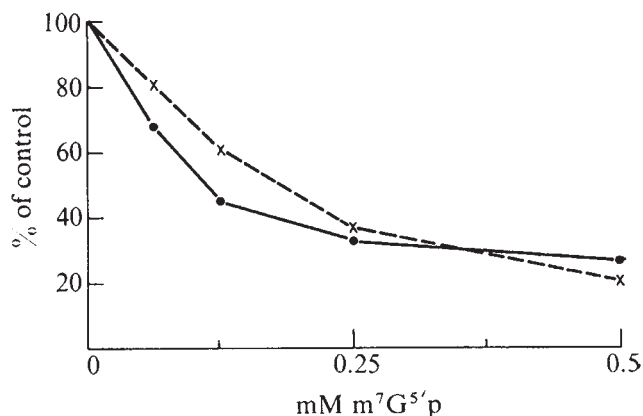
shown to contain a 7-methylguanosine residue linked, by way of a triphosphate, to the 5'-hydroxyl of the adjacent nucleotide ( $m^7G^{5'}ppp^5Xp$ ) (ref. 17). Removal of the 5'-terminal  $m^7G^{5'}p$  from globin mRNA prevents its translation<sup>18</sup>. Moreover, Both *et al.*<sup>19</sup> have shown that the presence of the methyl group in the 5'-terminal guanosine is required for translation of some viral RNAs. These RNAs can be obtained without a methyl group in the 5'-terminal guanosine by synthesising them *in vitro* in the presence of an inhibitor of methylation, S-adenosylhomocysteine (SAH)<sup>20</sup>. Wheat germ extracts can methylate these RNAs specifically to yield the structure  $m^7G^{5'}ppp^5G$  in the case of reovirus RNA, which can then be translated<sup>19</sup>. In the presence of SAH, however, the RNA is not methylated and cannot be translated<sup>19,20</sup>. Both *et al.*<sup>19</sup> considered the possibility that methylation may be involved in the activation of maternal mRNA at fertilisation.

The assay used so far to translate maternal mRNA could not discriminate between mRNAs methylated in the 5'-terminal



**Fig. 1** Stimulation of protein synthesis in a wheat germ cell-free system by RNA isolated from unfertilised sea urchin eggs. Eggs were collected from *Lytechinus pictus* by injection of KCl and washed as described previously<sup>8</sup>. About 10 ml of pelleted eggs were homogenised at 0 °C with 10 strokes of a loose fitting Dounce homogeniser in 35 ml of a buffer containing 200 mM NaCl, 5 mM magnesium acetate, and 20 mM Tris, pH 7.6. A post-mitochondrial fraction was obtained by centrifugation at 17,000g for 15 min. This fraction was adjusted to contain 1% sodium dodecyl sulphate and 10 mM EDTA, then warmed to room temperature and deproteinised with phenol-chloroform-isoamyl alcohol (25:24:1) (ref. 28). RNA was recovered by ethanol precipitation and separated into poly(A)+ and poly(A)- fractions by chromatography on oligo(dT)-cellulose<sup>22</sup>. A portion of the poly(A)- RNA was further fractionated by sucrose gradient centrifugation. The RNA sedimenting in the 6–12S region of the gradient was recovered by ethanol precipitation. All RNA preparations were reprecipitated from ethanol three times before translation in a wheat germ cell-free system<sup>21</sup>. Each assay contained in 25  $\mu$ l: 7.5  $\mu$ l of wheat germ extract, 1.2 mM ATP, 0.25 mM GTP, 15 mM creatine phosphate, 0.34 units of creatine phosphokinase (Sigma), 22 mM HEPES-KOH buffer pH 7.1, 3 mM magnesium acetate, 0.1 M KCl, 2.6 mM DTT, 1.8 mM mercaptoethanol, 50  $\mu$ M unlabelled amino acids minus lysine, and 2.5  $\mu$ Ci of  $^3$ H-lysine (38 Ci mmol<sup>-1</sup>). RNA was added to give the concentrations indicated. The incubation was carried out for 60 min at 24 °C and 5- $\mu$ l samples were taken in duplicate for counting as described previously<sup>21</sup>. A background of 800 c.p.m. obtained in an incubation without added RNA was subtracted from each value. Nonspecific stimulation resulting from the addition of ribosomal RNA (rRNA) was monitored by the addition of 16S RNA isolated from *E. coli* ribosomal subunits by phenol extraction and sucrose gradient centrifugation.  $\times$ , Poly(A)+ (total);  $\circ$ , poly(A)- (6–12S);  $\bullet$ , poly(A)- (total);  $\blacktriangle$ , *E. coli* 16S rRNA.





**Fig. 2** Inhibition by  $m^7G^{5'}p$  of the translation of poly(A)+ RNA isolated from unfertilised sea urchin eggs (●) and HeLa cells (×). The incubations were carried out as described in Fig. 1. Sea urchin egg poly(A)+ RNA was added to give  $95 \mu g \text{ ml}^{-1}$  and HeLa poly(A)+ RNA to give  $36 \mu g \text{ ml}^{-1}$ . Translation is expressed as a percentage of c.p.m. of incubations carried out in the absence of inhibitor. These values were 10,700 c.p.m. for sea urchin and 11,900 c.p.m. for HeLa RNA when corrected for the endogenous protein synthesis of the wheat germ extract.

guanosine and unmethylated mRNAs because of the endogenous methylating activity of the wheat germ extract already discussed. We have thus translated maternal mRNA in the presence of SAH to prevent its methylation during cell-free translation. In subsequent experiments we used a specific inhibitor of the translation of mRNAs with the 5'-terminus  $m^7G^{5'}ppp$  (ref. 21) to show that all the maternal mRNAs translated in a wheat germ cell-free system already have this 5'-terminal sequence before fertilisation.

Eggs of *Lytechinus pictus* were homogenised and RNA prepared as described in the legend of Fig. 1. The RNA was fractionated into polyadenylated (poly(A)+) and non-polyadenylated RNA (poly(A)-) by chromatography on oligo(dT)-cellulose<sup>22</sup>. The poly(A)+ RNA stimulated protein synthesis 10–15 times over the endogenous level of the wheat germ extract. Somewhat greater stimulation is obtained with poly(A)+ RNA from HeLa cells (Fig. 2)<sup>21</sup>. The poly(A)- RNA stimulates protein synthesis much less than the poly(A)+ RNA as a result of the large amount of non-translatable ribosomal and transfer RNA in this fraction. This stimulation is, however, consistently higher than that obtained with *E. coli* 16S ribosomal RNA (Fig. 1). Better stimulation of protein synthesis is obtained by translating the fraction of poly(A)- RNA which sediments between 6S and 12S during sucrose gradient centrifugation (Fig. 1). This fraction is enriched for histone mRNA<sup>23</sup>.

Translation of egg RNA is not affected by the presence of SAH (Table 1), indicating that methylation is not required for the translation of this RNA. On the other hand, addition of  $m^7G^{5'}p$  inhibits translation of poly(A)+ sea urchin RNA to the same extent as poly(A)+ RNA from HeLa cells (Fig. 2). This

latter RNA has been shown to have the 5'-terminal sequence  $m^7G^{5'}ppp$  (ref. 24). We have recently shown that the translation of all HeLa poly(A)+ RNA species is equally inhibited by  $m^7G^{5'}p$ , by analysing their translation products synthesised in the presence of inhibitory concentrations of  $m^7G^{5'}p$  (ref. 25). This nucleotide has no effect on the translation of satellite necrosis virus RNA in the wheat germ cell-free system<sup>21</sup>, or of encephalomyocarditis virus RNA in an HeLa cell-free system<sup>25</sup>. Neither of these viral RNAs has the 5'-terminal sequence  $m^7G^{5'}ppp$  (see ref. 25 for references).

The experiments reported rule out methylation of mRNA as a significant mechanism for the increased rate of protein synthesis at fertilisation of sea urchin eggs. In addition, it is unlikely that a 5'-terminal guanosine, which already contains a methyl group, is added to egg mRNA during the incubation in the wheat germ cell-free system. The reovirus RNA synthesised *in vitro* consists of a mixture of molecules with the 5' termini  $m^7G^{5'}ppp^5'G$ ,  $G^{5'}ppp^5'G$ , and  $pp^5'G$  (ref. 20). Both *et al.*<sup>20</sup> have shown that only those molecules which already contain the  $m^7G^{5'}ppp^5'G$  termini can bind to ribosomes and be translated by the wheat germ cell-free system in the presence of SAH. Therefore, the observation that egg mRNA is translated equally well in the presence or absence of SAH suggests that this RNA already contains the 5'-terminal  $m^7G^{5'}ppp$  sequence found in most eukaryotic mRNAs. This is supported further by experiments showing that the translation of maternal poly(A)+ RNA is inhibited by  $m^7G^{5'}p$  (Fig. 2).

We suggest that chemical modification of maternal mRNA does not have a significant role in the activation of protein synthesis at fertilisation. Maternal mRNA exists in the egg as ribonucleoprotein complexes<sup>8</sup>. The activation of protein synthesis at fertilisation may be a result of changes in the macromolecules associated with mRNA. Alternatively, the increased rate of protein synthesis may simply reflect the general metabolic activation of the zygote. A state of low respiratory activity occurs at the end of sea urchin oogenesis<sup>26</sup>, although unfertilised eggs contain concentrations of ATP and GTP as great as embryos<sup>27</sup>. It has been proposed that cell-surface macromolecules are involved in maintaining the low metabolic state of unfertilised eggs<sup>28</sup>. Fertilisation leads to an increased rate of overall cellular metabolism, which can support an increased rate of protein synthesis. If this latter explanation is correct, it should be possible to isolate ribonucleoproteins from unfertilised eggs and translate them *in vitro*. We are at present carrying out further studies designed to test this possibility.

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**Table 1** The effect of S-adenosylhomocysteine (SAH) on translation of sea urchin egg RNA

| RNA                 | Concentration<br>( $\mu g \text{ ml}^{-1}$ ) | $^3\text{H}$ -lysine incorporation (c.p.m.) |                |
|---------------------|----------------------------------------------|---------------------------------------------|----------------|
|                     |                                              | Control                                     | +0.32 mM SAH   |
| Poly(A)+<br>(total) | 95                                           | 9,455 (8.6 ×)*                              | 10,589 (8.0 ×) |
| Poly(A)-<br>(6–12S) | 400                                          | 6,173 (3.9 ×)                               | 6,958 (4.0 ×)  |

Experimental procedures are described in the legend of Fig. 1. The concentration of RNA is the final concentration in the assay mixtures. The concentration of SAH used (0.32 mM) inhibits the methylation of unmethylated reovirus RNA, which is required for its translation<sup>19</sup>.

\*Figures in parentheses indicate stimulation over endogenous incorporation (stimulation = (c.p.m. + RNA)/(c.p.m. - RNA)).

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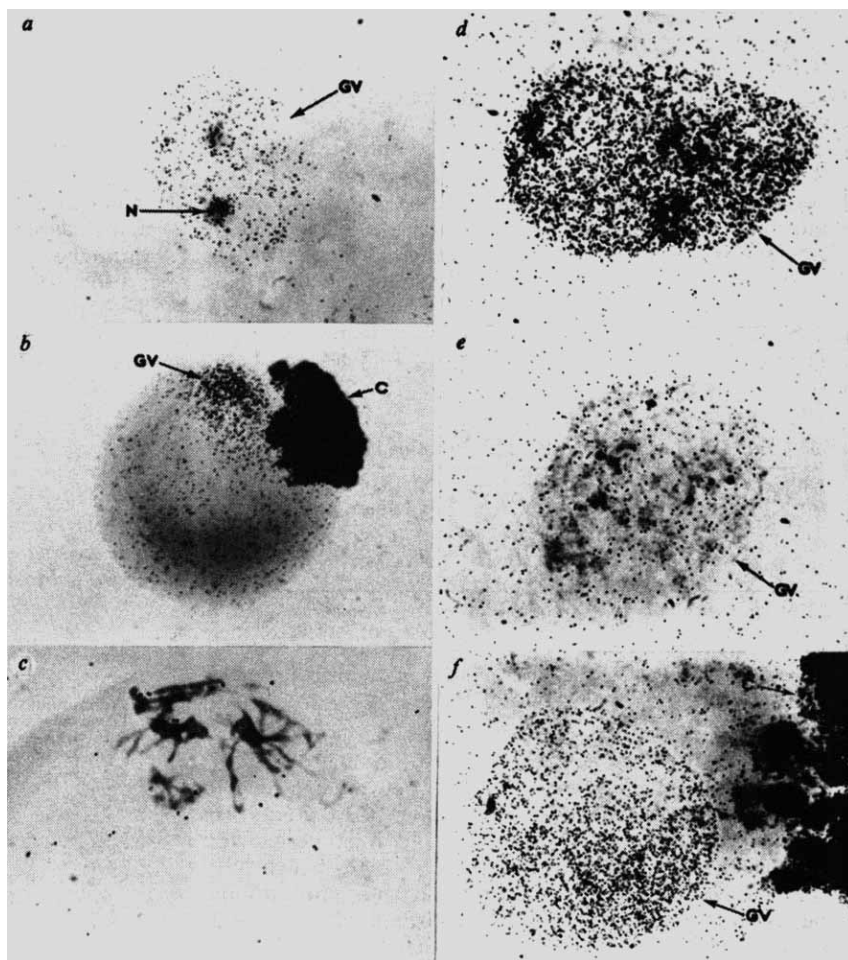
## RNA synthesis in fully-grown mouse oocytes

It is now well documented for various species that the progress of early development depends to a large extent on materials synthesised and stored in the oocyte during oogenesis. A well studied example of this phenomenon is

the synthesis and accumulation of both ribosomal and "informational" RNA in amphibian oocytes<sup>1-3</sup>. The extent of the contribution of oocyte RNA to early mammalian development is, however, not completely clear, since mouse embryos initiate rRNA synthesis at least as early as the four-cell stage of embryogenesis<sup>4,5</sup> and, unlike certain lower forms, the progress of early cleavage in the mouse is interfered with by inhibitors of RNA synthesis<sup>6-8</sup>. On the other hand, it is clear that growing mouse oocytes synthesise and store RNA<sup>9-12</sup>, such that the ovulated egg contains approximately the same amount of RNA on a volume basis as the amphibian egg (calculated from refs 13 and 14).

Shortly after birth, the mouse ovary is populated with thousands of small oocytes arrested in late prophase ('dictyate') of meiosis. Commencement of oocyte growth is apparently regulated within the ovary, the number of oocytes entering the growth phase being a function of the size of the pool of non-growing oocytes<sup>15,16</sup>. The oocyte and its surrounding follicle grow coordinately, progressing through a series of definable morphological stages<sup>17</sup>. The oocyte completes its growth in the adult mouse before the formation of the follicular antrum; consequently, the

**Fig. 1** Autoradiographs of mouse oocytes isolated from antral follicles micro-injected with <sup>3</sup>H-uridine or of mouse oocytes cultured *in vitro* with and without cumulus cells in the presence of <sup>3</sup>H-uridine. *a-c*, Follicles were dissected manually from the ovaries of adult (8-12 weeks of age), randomly bred, female Swiss mice (CD-1, Charles River Laboratories) and maintained in a chemically-defined culture medium<sup>24</sup>. The size of individual follicles was measured with an ocular micrometer attached to an inverted phase microscope. Each follicle was transferred, together with a small amount of medium, to a depression in an agar plate using a glass suction micropipette. A glass puncture micropipette attached to a Sensauer micro-manipulator was used to inject approximately 1.0 nl of <sup>3</sup>H-uridine (10 mCi ml<sup>-1</sup>, 26.2 Ci mmol<sup>-1</sup>, New England Nuclear), made-up in culture medium, into each follicle. Follicles were then transferred through two washes of medium and cultured for 1 h at 37° C in an humidified atmosphere of 5% CO<sub>2</sub> in air. The entire injection and wash procedure required approximately 1 min for each follicle treated. At the end of the culture period, follicles were physically disrupted individually and the oocytes were placed on albumin-coated slides, cover slipped, and fixed by drawing a solution of alcohol-acetic acid (3:1 by volume) through. The slides were then frozen, cover slips flicked off, and the slides washed and coated with Kodak NTB 2 liquid emulsion. Slides were stored in the dark at 4° C for 5 d and were developed using standard procedures. In some cases preparations were stained with Giemsa (Harleco No. 620) at a 1:50 dilution with 0.1 M phosphate buffer, pH 6.8, for 30 min and/or treated with ribonuclease (0.1%, Sigma) for 4 h at 37° C. Light microscopy was performed using a Zeiss Photomicroscope II. *a*, Oocyte from antral follicle, approximately 500 µm in diameter; *b*, Oocyte from antral follicle, approximately 450 µm in diameter, with adhering cumulus cells; *c*, Oocyte from antral follicle, approximately 500 µm in diameter, which has undergone germinal vesicle breakdown and chromosome condensation. *d-f*, Oocytes were obtained by puncturing ovaries from adult Swiss mice<sup>25</sup>. Oocytes containing an intact germinal vesicle, freed of or surrounded by cumulus cells, were collected using a mouth-operated micropipette and washed in culture medium. Cell culture was carried out in embryological watch glasses in medium containing <sup>3</sup>H-uridine (50 µCi ml<sup>-1</sup>, 26.2 Ci mmol<sup>-1</sup>, New England Nuclear) under paraffin oil at 37° C in a humidified atmosphere of 5% CO<sub>2</sub> in air. Oocytes were cultured for 2 h, collected and washed, and those cultured in the presence of cumulus cells were denuded by repeated pipetting. The oocytes were then treated for autoradiography as above. *d*, Oocyte cultured in the presence of cumulus cells; *e*, Oocyte cultured in the absence of cumulus cells; *f*, Oocyte cultured in the presence of cumulus cells, with adhering cumulus cells. GV, germinal vesicle; N, nucleolus; C, cumulus cells.





majority of follicle growth occurs after the oocyte has stopped growing<sup>18</sup>. This report shows that mouse oocytes continue to synthesise RNA even during the period of follicular antrum formation when oocyte growth has ceased, a finding which differs with the current view that RNA synthesis in mouse oocytes is inhibited at the time of, and subsequent to, antrum formation<sup>9,11</sup>.

We find that when <sup>3</sup>H-uridine is administered intraperitoneally to adult mice, and its incorporation into oocyte RNA is evaluated soon after by autoradiography of ovarian sections, there is a positive correlation between oocyte size and uridine incorporation within the oocyte's nucleus (germinal vesicle) in follicles lacking an antrum. Those follicles containing an incipient or highly developed antrum display virtually no grains over the oocyte's germinal vesicle or nucleolus. These observations are essentially identical to those of several other investigators and have led previous workers to conclude that at the time of antrum formation RNA synthesis within the oocyte is suppressed<sup>9,11</sup>. This means that for a period of several days, while the follicle undergoes tremendous growth, the fully-grown oocyte possessing an intact germinal vesicle and nucleolus is quiescent with respect to RNA synthesis. Alternatively, these observations could reflect, not the absence of RNA synthesis in oocytes of antral follicles, but rather, a precursor pool of UTP of such low specific activity that oocyte RNA synthesis goes undetected. A decrease in the permeability of the follicle and/or oocyte to exogenous <sup>3</sup>H-uridine, expansion and/or compartmentalisation of the endogenous uridine pool of the oocyte, or any of a number of other changes could lead to a decrease in the specific activity of the oocyte's UTP pool at about the time of antrum formation.

To explore the possibility that oocytes within antral follicles actually continue to synthesise RNA, we have injected <sup>3</sup>H-uridine directly into antral follicles (450-500  $\mu$ m in diameter) dissected from mouse ovaries, incubated the follicles for a short time in chemically defined medium, and then isolated and examined the oocytes autoradiographically. Although we do observe lower incorporation of uridine in oocytes isolated from antral follicles compared

pipetting show only low levels of uridine uptake and incorporation, oocytes surrounded by cumulus cells show increased uptake of uridine and heavy incorporation over the oocyte's germinal vesicle and especially over the nucleolus (Fig. 1 and Table 1). These experiments not only point to the importance of interactions between oocytes and cumulus cells, at least in so far as the entrance of exogenous uridine into the oocyte's intracellular pool is concerned, but also demonstrate clearly that fully-grown oocytes isolated from antral follicles synthesise RNA as long as there is an intact germinal vesicle and nucleolus. In experiments not presented here (Wassarman and Letourneau, unpublished), we find that RNA synthesis ceases concomitant with the onset of meiotic maturation of the oocyte, which includes dissolution of both the germinal vesicle and nucleolus.

The findings reported here are of particular interest in connection with studies of RNA synthesis during oogenesis in *Xenopus laevis*. For some time it was considered that fully-grown amphibian oocytes are quiescent with respect to RNA synthesis<sup>19-21</sup>, attributable, perhaps, to the presence of a specific inhibitor of rRNA synthesis<sup>22,23</sup>. Recent work has, however, shown that these oocytes are not metabolically dormant, but synthesise total RNA at a rate at least as great as smaller oocytes<sup>13</sup>. Two factors which contributed to the impression that fully-grown oocytes do not synthesise RNA are a greatly reduced uptake of exogenous precursors by fully-grown, compared with smaller, oocytes and expansion of the oocyte's endogenous precursor pool which accompanies oocyte growth; both factors can lead to low or undetectable levels of incorporation of radioactive precursors into RNA. The observations reported here suggest that similar factors have influenced the results of previous studies of RNA synthesis during oogenesis in the mammal and have led to an incorrect assessment of the actual situation. Mouse oocytes actively synthesise RNA throughout the period of follicle growth and terminate synthesis only at the time of germinal vesicle and nucleolus dissolution.

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**Table 1** Uptake and incorporation of <sup>3</sup>H-uridine into mouse oocytes

| Culture time (h) | C.p.m./Oocyte*  |                | Grains/GV†     |              |
|------------------|-----------------|----------------|----------------|--------------|
|                  | +Cumulus        | -Cumulus       | +Cumulus       | -Cumulus     |
| 1.0              | 16.0 $\pm$ 2.1  | 10.2 $\pm$ 2.2 | 320 $\pm$ 55   | 110 $\pm$ 40 |
| 2.0              | 62.6 $\pm$ 14.8 | 9.8 $\pm$ 1.9  | 1550 $\pm$ 450 | 280 $\pm$ 60 |

\* Determination of TCA-soluble <sup>3</sup>H-uridine was carried out as previously described<sup>26</sup>. The mean  $\pm$  s.e.m. for a total of 50 or more oocytes is shown.

† These values represent the mean number of grains per germinal vesicle for 20 oocytes. The values do not include the nucleolar area of the germinal vesicle.

with preantral follicles, it is clear that oocytes continue to synthesise RNA throughout follicular growth; grains are found concentrated within the oocyte's germinal vesicle and nucleolus (Fig. 1). On the other hand, oocytes which have undergone germinal vesicle dissolution and chromosome condensation within the follicle show virtually no incorporation of <sup>3</sup>H-uridine (Fig. 1). Assuming that the rate of RNA synthesis in oocytes within isolated follicles is the same as that *in vivo*, these results suggest that previous investigations, which involved injection of <sup>3</sup>H-uridine into the animal, rather than into isolated ovarian follicles, failed to detect RNA synthesis in oocytes of antral follicles as the result of precursor pools with too low a specific activity to enable measurement. Additional support for this suggestion comes from experiments carried out with isolated mouse oocytes cultured *in vitro* in the presence of <sup>3</sup>H-uridine. Although oocytes freed of cumulus cells by repeated

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# matters arising

## Spreading rate in Iceland

WALKER has proposed a new interpretation of Icelandic tectonics in which he recognises the existence of a number of parallel or *en echelon* rift zones<sup>1</sup>. He showed that the aggregate width of the active zones is greatest in southern Iceland and, by postulating that this width is proportional to spreading rate, inferred that the spreading rate in southern Iceland may be three to four times faster than in northern Iceland or the adjacent mid-ocean ridges.

There is one serious objection to this idea. An excess spreading rate in the southern part of Iceland would generate a greater length of lithospheric plate, and either this would have to be taken up in a zone of plate consumption, or else it would result in major compressive deformation of at least one of the plates. There is no evidence that either effect occurs, and I suggest the following explanation of the observations.

The increased widths of young rock outcrops and the greater number of active volcanic centres and thermal fields in the south of Iceland, cited by Walker in support of his hypothesis, are in fact very good evidence of increased magmatism, though they do not necessarily imply an increased spreading rate. The excess material produced can be accommodated by crustal thickening rather than accelerated spreading, thus avoiding the plate-tectonic complications mentioned above. There is abundant geophysical evidence that there is in fact a thickened crust under Iceland as a whole<sup>2</sup>, though it is not clear whether the thickening increases towards the south. It seems quite possible that the resultant thicker lithosphere would distribute tectonic stresses over a wider area, giving rise to a pattern of rift zones such as that observed by Walker, and obviating the need to assume that the width of the active zones is proportional to spreading rate.

The same reasoning may be applied to Afar and the Azores, each of which has an excessively thick crust<sup>3,6</sup> and complex rift zones (ref. 4 and D. C. Krause and B. A. McGregor, unpublished) analogous to those described for Iceland. Schilling<sup>5</sup> has proposed that the thicker crusts in all three areas arise from excess magmatism generated by mantle blobs or plumes, and Walker's observations of increased magmatism in southern Iceland repre-

sent a partial confirmation of that, and suggest that it is in southern Iceland that the present plume discharge is centred.

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WALKER REPLIES—I fully accept the awkward implications of excess spreading in the southern part of Iceland, as raised by Searle. Some excess spreading could, however, be accommodated by an outward bulging of this part of Iceland, but a determination of its extent must await the delineation of magnetic strip anomalies around the east of the country. Some excess material produced by volcanism could be accommodated by the observed crustal thickening.

An alternative possibility is that basalt is destroyed on a large scale low down in the Icelandic crust, and this is supported by a significant anomaly in the geology of Iceland. This anomaly, the existence of which has apparently not hitherto been pointed

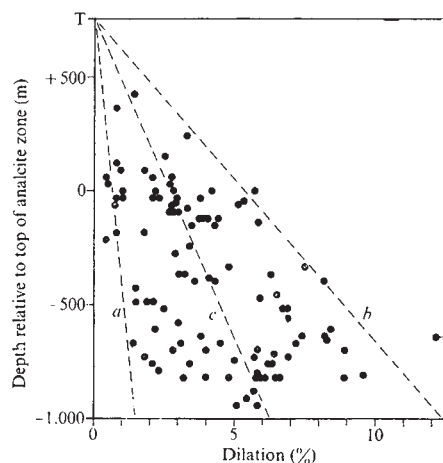
out, is the low dyke intensity gradient in the Icelandic dyke swarm. It should be explained that quantitative studies of the Tertiary swarm have been made at many sites in the fjordlands of eastern Iceland<sup>1,2</sup>, and everywhere reveal that the dyke intensity increases linearly downwards. On Fig. 1 all the best dyke intensity data, quoted as a percentage dilation, are plotted against depth relative to the top of the analcite zone; this zeolite zone boundary is the most convenient datum level, and is believed to lie 750 m below the original (pre-erosion) top of the Tertiary crust. Much scatter appears on Fig. 1, because at any one level the dyke swarm varies in intensity according to the position of the measuring site relative to local sub-swarms. The intensity gradient varies between 1% dilation per 140 m and 1% per 1,100 m depth, and the median gradient is ~ 1% per 280 m depth.

A requirement of crustal spreading by the conventional dyke injection model is that the dyke swarm intensity should attain 100% at the base of the crust. Extrapolation of the measured intensity gradient yields 100% dilation at a depth between 14 and 110 km, or 28 km if the median gradient is taken. The crustal thickness is not known for eastern Iceland, but from similar areas elsewhere in Iceland is likely to be ~ 15 km (refs 3, 4). A discrepancy therefore clearly exists: if the median intensity gradient is taken, then > 10 km of crust would seem to be missing.

The magnitude of the discrepancy is critically dependent on the nature of crustal layer 3: whether it consists of metamorphosed basalts<sup>3</sup>, or is largely made of intrusive sheets<sup>5</sup>. Layer 3, the lowermost crustal layer, with a P-wave velocity averaging 6.5 km s<sup>-1</sup>, is found beneath the whole of Iceland and in eastern Iceland occurs on average ~ 3 km below sea level (or 4 km below the original top of the crust). At the top of layer 3 the extrapolated dyke intensity is between 3.6 and 29%, or 14% taking the median gradient. If layer 3 consists largely of an intrusive sheet swarm, then the basaltic lavas in the layer are in effect greatly attenuated by the sheets and the discrepancy is therefore greatly increased. If the median gradient is taken, then several tens of kilometres of crust may be missing.

There is another relationship which seems to support the same general con-

**Fig. 1** Plot of variation in percentage dilation of the dyke swarm against depth in the Tertiary lava pile of eastern Iceland, relative to the top of the analcite zeolite zone. Each dot is based on the measurement of dyke thicknesses in a strip of well-exposed country on average 1 km long. *a*, 1% dilation per 1,100-m gradient; *b*, 1% per 140-m gradient; *c*, 1% per 280-m gradient.





clusion. It depends on how the total width,  $D$ , of dykes injected in a rift zone compares with the total thickness,  $L$ , of lava flows produced within the same time period. Crustal spreading models assume that  $L=D$ , which is the ideal situation for steady-state spreading. From the author's experience of volcanism in Iceland it seems more likely, however, that  $L > D$ , since the typical eruption produces lava totalling tens of metres thick near source, compared with a width of 2 or 3 m for the dyke feeder. An imbalance still remains when dykes in non-eruptive fissures are included.

There is no *a priori* reason why  $L$  and  $D$  should be equal;  $L$  is controlled more by the thermal regime in the mantle immediately below Iceland, whereas  $D$  is controlled more by the global stress system, although there is some latitude for a local increase by outbulging and other readjustments within Iceland. If  $L > D$ , then the crust must inevitably thicken with time. The fact that the Icelandic crust does not thicken in this manner indicates the operation of a counteractive effect destroying crustal material.

Two types of destruction can be envisaged. One type is a general 'ablation' or sapping of material from the base of the crust; the generation of acid magmas by the partial fusion of basalt may be a byproduct of it. The other type is a periodic foundering of big crustal blocks into the upper mantle. The average P-wave velocity in the upper mantle beneath Iceland is only 10% higher than that in crustal layer 3, and the density contrast is likely to be of comparable magnitude. In some circumstances a higher degree of partial melting in the upper mantle might so reduce its density as to cause a density inversion to arise, such that a large scale foundering of layer 3 becomes possible.

There are two prerequisites for the development of a density inversion, namely an appropriate buildup of heat in the upper mantle, and an efficient cooling of the crust so as to preserve its relatively high density. Regarding the latter, the nature of magmatism in Iceland is such as to generate rapidly great volumes of cold rock in the upper part of the crust within and near the active rift zones, and to depress these cold rocks rapidly towards the mantle. The cold rocks consist partly of lava flows which cool rapidly in the air, and partly of dykes intruded above layer 3 or sheets intruded at the top of layer 3, where water cooling by circulating groundwater is extremely effective. Viewed broadly, a high heat loss rate from the Icelandic mantle hot spot is achieved by three main mechanisms besides conductive heat flow: the outpouring of lavas, the emplacement and

water cooling of high level minor intrusions, and the rapid down-sagging of cold crustal rocks towards, and perhaps their incorporation in, the mantle.

One can speculate that a major foundering episode took place in the southern half of Iceland between 3 and 5 million years ago, and was responsible for a major episode of crustal flexuring (that which formed for example the Lagarfljót flexure zone in eastern Iceland<sup>2</sup>), and for the birth of some of the multiplicity of rift zones in the resulting thinned crust of southern Iceland<sup>6</sup>, besides perhaps facilitating outbulging to accommodate the increased spreading there.

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## Aromatic structures in coal

THE demonstration by Hayatsu *et al.*<sup>1</sup> that dichromate oxidation of coal allows isolation of clearly identifiable hetero-aromatic entities is important because very little is so far known about such structures in coal. But it does not by any means invalidate our inference<sup>2</sup> that the skeletal structure of coal is predominantly non-aromatic, and is, in fact, entirely consistent with that view.

Bearing in mind how susceptible coal is to oxidation by air at temperatures as low as 70 °C, autoclaving it with aqueous dichromate at 250 °C for 36 h is hardly a mild treatment, and raises the distinct possibility of pyrolytic cleavages and major structural rearrangements. We note, in this connection, that there is a large body of evidence that coals other than anthracites begin to suffer substantial thermal decomposition well below 200 °C.

Assuming that the carboxylic acids identified by Hayatsu *et al.* did not form pyrolytically set up polynuclear 'artefacts', considerable interest attaches to the carbon balance which quantifies their findings. From the nature and yield of their oxidation products

we calculate that at most 45% of the original carbon in the coal was recovered in the carboxylic acids. The remainder was lost to CO<sub>2</sub> and since dichromate does not, as Hayatsu *et al.* correctly point out, cause significant ring degradation, this must have come from non-aromatic (Sp<sup>3</sup> carbon) structures. Moreover, since the carboxylic moieties must, for the same reason, also have formed from aliphatic and/or alicyclic linkages, at least 25% of the carbon in the carboxylic acids must likewise be assigned to the Sp<sup>3</sup> valence state. Overall, then, the results obtained by Hayatsu *et al.* indicate that some 65% or so of the total carbon in coal exists in the Sp<sup>3</sup> state—which coincides almost exactly with our estimates from hypohalite oxidation of coal.

It appears that all coal oxidation studies, regardless of what oxidants and reaction conditions are used<sup>3–5</sup>, lead to substantially similar products and strikingly similar Sp<sup>3</sup>:Sp<sup>2</sup> carbon ratios (see Table 1). Were one to assume that coal is mostly comprised of polycyclic aromatic structures, one would therefore be forced to conclude that weak oxidants, such as sodium hypochlorite ( $E_B^0=0.89$ ) or dichromate ( $E_B^0=1.2$ ) can oxidise polycondensed aromatics as readily as alkaline potassium permanganate ( $E_B^0=1.69$ ) or concentrated nitric acid—a conclusion that is inconsistent with what Hayatsu *et al.* correctly designated for dichromate oxidation. If nothing else, the sensitivity of coal to oxidation and the fact that the strength of the oxidant is not a prime determinant of the vigour of the reactions should be enough to suggest a predominantly non-aromatic structure. Relatively asymmetrical, branched 'diamondoid' skeletal structures, which are much more easily oxidisable than polycondensed aromatics with conserved symmetry, would certainly be more consistent with experimental observations.

In these circumstances, we conclude that discovery of C<sub>10</sub>-, C<sub>14</sub>- and C<sub>18</sub>-polycyclics among the oxidation products of coal, if confirmed by less drastic means than those used by Hayatsu *et al.*, only requires a review of earlier reports that oxidation never delivers anything beyond single-ring compounds (and CO<sub>2</sub>).

**Table 1** Distribution of carbon in oxidation products (as percentage of coal carbon, Pittsburgh seam)

| Products         | Alkaline KMnO <sub>4</sub><br>(various conditions) | NaOH + H <sub>2</sub> O + O <sub>2</sub><br>Press 7–25 atm<br>100–250 °C | Na <sub>2</sub> Cr <sub>2</sub> O <sub>7</sub> + H <sub>2</sub> O<br>Autoclave<br>250 °C | NaOCl + H <sub>2</sub> O<br>60–65 °C<br>Open flask |
|------------------|----------------------------------------------------|--------------------------------------------------------------------------|------------------------------------------------------------------------------------------|----------------------------------------------------|
| Carbonate carbon | 45.0                                               | 50.0                                                                     | 65.0                                                                                     | 63.0                                               |
| Oxalate carbon   | 15.0                                               | 13.0                                                                     |                                                                                          |                                                    |
| Aromatic carbon  | 31.0                                               | 36.0                                                                     |                                                                                          |                                                    |
|                  |                                                    |                                                                          | 35.0                                                                                     | 37.0                                               |

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HAYATSU ET AL. REPLY—First, with respect to the ease of oxidation of coal by air at 70 °C we need only quote from ref. 1: "For example, at 180 °C and oxygen concentrations <20%, conversion to humic acids is negligible, and significant reaction rates at this temperature are only observed when [O] >50%".

Detailed pyrolysis studies of the bituminous coal we used for our experiments showed no evidence of thermal decomposition until the coal was heated well above 250 °C.

Second, the estimate of the degree of aromatic structure from our data is much too low for the following reasons: (a) Because of the large concentrations of salts resulting from oxidation with Na<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub>, yields were not quantitative and were estimated to be ~75%. As we mentioned in our paper the amphoteric acids were not isolated from this oxidation but were identified in the product from a photochemical oxidation with much less interfering residues; (b) a significant fraction of the aromatic compounds appeared in a neutral fraction, and (c) as Friedman has shown, aromatic rings containing phenolic groups would have been destroyed by Na<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub>.

By a method based on the stoichiometry of fluorination reactions (J. Huston, R. G. Scott, and M. H. Studier, unpublished) we have calculated the aromaticity to be 69%.

Third, we agree that the strength of an oxidising agent is less important than its specificity in studying coal structure. Friedman<sup>2</sup> has demonstrated the selectivity of aqueous sodium dichromate in an autoclave at 250 °C without pyrolytic cleavage or structural rearrangements occurring. Several authors<sup>3-5</sup> have shown the non-specificity of aqueous NaOCl, the reactions of which are the basis of Chakrabarty's and Berkowitz's postulate that coal is primarily non-aromatic.

We feel it more likely that coal consists of aromatic units bridged largely by aliphatic and hydro-aromatic groups than that it has a 'diamondoid' structure.

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- <sup>1</sup> Jensen, E. J., Melnyk, N., Wood, J. C., and Berkowitz, N., *Adv. Chem. Ser.*, **55**, 621 (American Chemical Society (1966)).

- <sup>2</sup> Friedman, L., Fishel, L., and Shechter, H., *J. org. Chem.*, **30**, 1453 (1965).
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## Trapped helium and argon and formation of the atmosphere

FISHER<sup>1</sup> reports 'He/<sup>40</sup>Ar ratios from analyses of the glassy margins of several deep sea basalts which are higher than can be generated by the *in situ* decay of K, U and Th in the mantle if one uses conventional estimates for the K/U and Th/U ratios in the mantle. He then concludes that "the data rule out all models which ascribe the terrestrial atmosphere to any type of degassing of a mantle which is chondritic or crustal with respect to the K/U ratio". While this sweeping conclusion may ultimately prove correct, Fisher's argument is suspect since it involves an assumption which is probably false. The assumption is explicitly stated: "The rare gases found in deep sea basaltic glasses are clearly those trapped within the rock on its eruption, due to the high hydrostatic pressures and rapid chilling, and as such represent mantle-ambient gases".

Oceanic basalt magmas are commonly taken to be fractionated (by partial melting) samples of the mantle. Ozima and Alexander (unpublished) have argued that the partial melting process produces large enrichments of light rare gases relative to the heavy rare gases in the melt phase. For example, Gramlich and Naughton<sup>2</sup> have shown that Iherzolite nodules, which they conclude represent unfused residue from the partial melting process, contain 'He/<sup>40</sup>Ar ratios lower than reasonable values. If the residue is depleted in 'He relative to <sup>40</sup>Ar, simple mass balance requires that the melt phase be enriched in 'He relative to <sup>40</sup>Ar. While data from the glassy rims do probably represent lower limits on the 'He/<sup>40</sup>Ar ratios in the magma, the magma values are only bad upper limits on the mantle-ambient values. The magma 'He/<sup>40</sup>Ar ratios do not, therefore, rule out the degassing models mentioned above.

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- <sup>1</sup> Fisher, D. E., *Nature*, **256**, 113-114 (1975).
- <sup>2</sup> Gramlich, J. W., and Naughton, J. J., *J. geophys. Res.*, **77**, 3032-3042 (1972).

FISHER REPLIES—The suggestion of Alexander<sup>1</sup> (based on unpublished work of Ozima and Alexander) that the 'He/<sup>40</sup>Ar ratio may be quantitatively enriched in the mantle-magma segregation process is certainly interesting, though unproven. Alexander's sugges-

tion that Gramlich and Naughton<sup>2</sup> concluded that their samples represent residue from the partial melting process with He depleted relative to Ar, and therefore that the melt phase must be enriched in He relative to Ar, ignores Gramlich and Naughton's actual interpretation of their data: that their gas ratios were probably "altered during a high temperature episode such as that existing during transport in the erupting lava".

We know that such a high temperature episode existed (the eruption itself), and that this would segregate He from Ar; therefore we cannot use these data as evidence for another and previous segregation process, as Alexander would like. The simplest interpretation of my data remains that the measured ratios represent those in the source material except as modified during eruption; that is, they are lower limits to the mantle-ambient ratios. Experimental investigations that might prove the reality of the Ozima-Alexander model will be welcomed.

Alexander (personal communication) has pointed out an error in Fig. 1 of my paper<sup>3</sup>: the values for all curves below 10<sup>7</sup> yr should be levelling off, not decreasing. This does not affect in any way the arguments presented either there or here.

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- <sup>1</sup> Alexander, E. C., Jr., *Nature*, **261**, 77 (1976).
- <sup>2</sup> Gramlich, J. W., and Naughton, J. J., *J. geophys. Res.*, **77**, 3032-3042 (1972).
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## Ambiguous cognitive contours

BRADLEY and Dumais<sup>1</sup> have presented figures that induce ambiguous subjective contours, which tend to alternate between two (or more) perceived forms. Bradley and Dumais suggested that because these figures are not "stimulus-bound", they cannot be accounted for by a physiological model, but arise when alternative perceptual hypotheses are tested<sup>2</sup>. Although this account is plausible, a more physiological explanation may also exist. The alternation of perceived forms resembles an effect described by Campbell *et al.*<sup>3,4</sup>: if two gratings that have sinusoidal luminance profiles are projected so that their axes are at an angle to one another, the gratings tend to be seen in alternation, one replacing the other at intervals of several seconds. Campbell *et al.* suggest that this occurs because while one grating is perceived, the channel that is most sensitive to that grating becomes adapted. When adapted, its sensitivity decreases<sup>5</sup>, allowing the other grating to predominate.

When observed steadily, Bradley and Dumais' patterns undergo a similar



alternation. This is seen most easily in their star pattern, in which the two triangles predominate alternately. It is difficult or impossible to maintain the perception of one triangle, although if they had a cognitive origin, one would expect to be able to do so. Furthermore, an adaptation hypothesis can better account for the fact that the figure that is predominant at a given moment appears brighter. If adaptation takes place, it is reasonable that the less strongly adapted figure should be brighter. It thus seems premature to conclude that this phenomenon cannot eventually be accounted for by a physiological model.

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<sup>1</sup> Bradley, D. R., and Dumais, S. T., *Nature*, 257, 582-584 (1975).

<sup>2</sup> Gregory, R. L., *Nature*, 238, 51-52 (1972).

<sup>3</sup> Campbell, F. W., Gilinsky, A. S., Howell, E. R., and Riggs, L. A., *Perception*, 2, 123-125 (1973).

<sup>4</sup> Atkinson, J., Campbell, F. W., Fiorentini, A., and Maffei, L., *Perception*, 2, 127-133 (1973).

<sup>5</sup> Blakemore, C., and Campbell, F. W., *J. Physiol., Lond.*, 203, 237-268 (1969).

BRADLEY, DUMAIS AND HEYWOOD M. PETRY REPLY.—If Cavonius is correct in attributing the alternating organisations of our ambiguous configurations to the adaptation of "the channel that is most sensitive to that grating" (ref. 1), then such configurations should alternate independently of the viewer's knowledge of the possible organisations. It is not difficult, however, to construct figures which are highly

resistant to alternations until the viewer is sensitised to the existence of alternative organisations. An example of such a figure, called the subjective Necker cube<sup>2</sup>, is presented in Fig. 1a. If observers are permitted to look at this figure for an extended period, most will eventually report seeing a cube overlying eight black disks and a white background (as in the unambiguous version of Fig. 1b). The whiter-than-white bars of the cube seen extending between the disks are, of course, illusory and are bounded by subjective contours.

Having achieved this cube-in-front organisation, most observers will maintain it indefinitely. If shown the unambiguous cube-in-back organisation of Fig. 1c, however, they then report being able to see the cube in Fig. 1a in that position as well, with subjective contours now bounding the interior edges of the 'holes' of the occluding surface rather than extending between the disks. Of course, once aware of these two organisations, the observer will then experience spontaneous alternation between the cube-in-front and cube-in-back. Although this might be accounted for by an adaptation hypothesis, such an hypothesis could not account for the initial absence of alternation. Furthermore, the observer can be 'cued' to a third possible organisation; that depicted in Fig. 1d. In this case, the observer will see both straight and curved subjective contours in Fig. 1a, because the 'straddled' organisation cued by Fig. 1d puts part of the cube in front and part in back. It seems

reasonable to suppose that perceptual phenomena which are strongly affected by set or expectation probably have a cognitive origin.

This view does not preclude the possibility of a physiological explanation of subjective contours. It does, however, require that any such explanation be able to incorporate the effects of cognitive variables (set, expectation, attention and so on) on the manner in which subjective contours are perceived in ambiguous displays.

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<sup>1</sup> Cavonius, C. R., *Nature*, 261, 77 (1976).

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## Insectivorous grouse

It is, perhaps, unfortunate that we did not publish the results of incubating hens and other adult grouse separately in our paper on insects as food of adult grouse<sup>1</sup>. In 1974, droppings from four incubating grouse on wet moors (at Moor House, Cumbria) were examined and contained similar proportions of insect remains (mainly tipulids) as did non-incubating birds. Similarly, females with young of different ages all showed tipulid remains in their droppings. Thus Moss and Watson<sup>2</sup> can rest assured that incubating and brooding female red grouse do, at least on occasion, feed on tipulids in addition to vegetation.

Moss and Watson go on to state "there is no evidence that tipulids are ever in short supply on wet moors". We have information to the contrary<sup>3-5</sup>. In addition, both *Molophilus ater* and *Tipula subnodicornis*, the main species involved, fluctuate markedly in numbers and larval densities between 2,500 m<sup>-2</sup> and 50 m<sup>-2</sup> in the former and 250 m<sup>-2</sup> and 5 m<sup>-2</sup> in the latter have been documented by us on several occasions.

The relationship between grouse numbers and tipulid densities on wet moors is unknown. Even the cautious research workers might consider investigating the possibility of such a relationship, particularly since it has been shown that N and P, which are in much higher concentrations in tipulids than in *Calluna*, are important to grouse<sup>6</sup>.

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<sup>1</sup> Butterfield, J., and Coulson, J. C., *J. Anim. Ecol.*, 44, 601 (1975).

<sup>2</sup> Moss, R., and Watson, A., *Nature*, 259, 250 (1976).

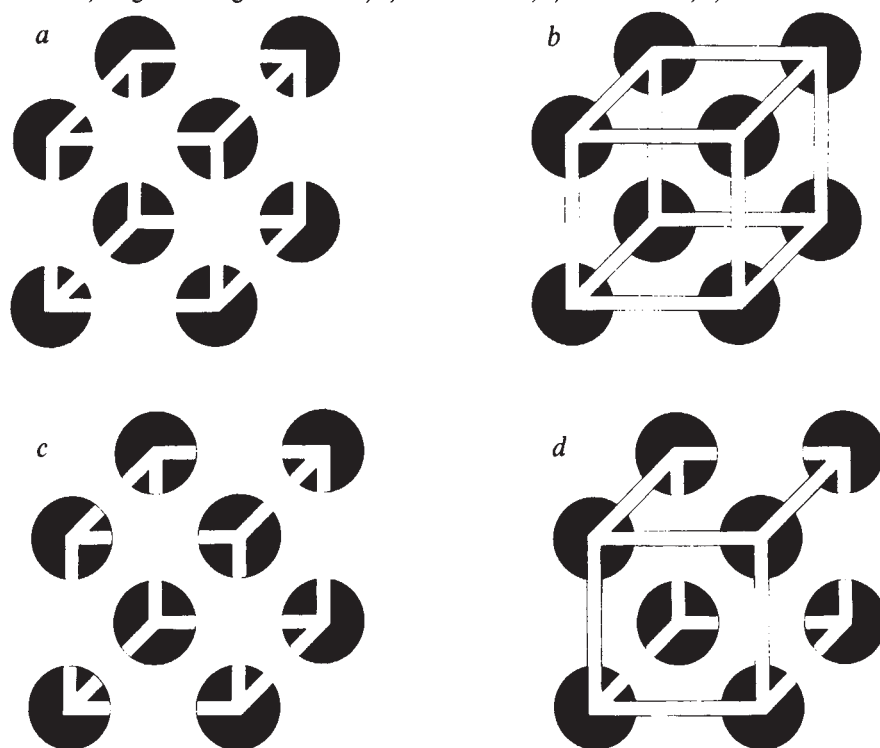
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<sup>5</sup> Coulson, J. C., and Whittaker, J. B., in *Ecology of Some Moors and Montaine Grasslands* (Springer, in the press).

<sup>6</sup> Moss, R., *J. Anim. Ecol.*, 41, 411 (1972).

Fig. 1 Unambiguous versions of three possible organisations of the subjective Necker cube. a, Original ambiguous version; b, cube-in-front; c, cube-in-back; d, straddled.



# reviews

## IBP—nitrogen fixation

Christina Kennedy

*Nitrogen Fixation by Free-Living Micro-organisms.* (International Biological Programme 6.) Edited by W. D. P. Stewart. Pp. xxi+471. £13.50. *Symbiotic Nitrogen Fixation in Plants.* (International Biological Programme 7.) Edited by P. S. Nutman. Pp. xxviii+584. £22.00. (Cambridge University: Cambridge, London and New York, 1976.)

AN outstanding feature of the IBP synthesis meeting on nitrogen fixation held in Edinburgh in September 1973 was its international character. That very strength created a potential weakness as far as publication of the proceedings was concerned, and I remember sometimes labouring over abstracts at that meeting wondering just what it was that I had come to learn about. Messrs Stewart and Nutman, the editors, are therefore to be congratulated on the high standard of English and easy readability of both these volumes which are largely based on that IBP meeting. Both volumes contain a mixture of review articles, original research papers and a few compiled results of surveys. The reader must beware, however, of chapter titles that belie their actual contents.

The first two sections of volume 6 give roughly equal time to the two major groups of free-living nitrogen fixers, certain bacteria and blue-green algae. The reports of field research in Brazil (Dobereiner and Day, ch. 3) and in the UK (the longstanding Broadbalk experiments at Rothamsted; Day *et al.*, ch. 5) are elegant examples of how to assess the ecological importance of bacterial nitrogen fixation, but this section would benefit from an overview of the relative importance of the various groups of free-living nitrogen-fixing bacteria. There is also very little about the regulation or physiology of fixation in bacteria other than azotobacters.

If we are left wondering about the overall contribution of non-symbiotic bacteria to the world's agricultural nitrogen balance, there can be little doubt about the usefulness of blue-green algae in certain agricultural situations. These are extensively covered in volume 6 and complemented by the excellent chapters in volume 7 on the symbiotic associa-

tions of blue-green algae with plants. It is a pity that all this work is not included in the same volume in spite of the different modes of fixation.

Volume 6 continues with a section on the use of acetylene reduction as a measure of nitrogen fixation which, although useful, is not the last word, as equally valuable descriptions of this technique are described throughout research articles in both volumes. The final biochemistry section contains some well-written, if outdated, reviews and research reports on nitrogenase (this faster-moving branch of nitrogen fixation has been often reviewed recently). Still timely is ch. 26 on reductants for nitrogenase. Hardy *et al.* remind us (ch. 24) that there is another world of relevant research on the chemistry of dinitrogen complexes.

Two chapters in the biochemistry section of volume 6 describe the serological identification of rhizobia and leghaemoglobin biosynthesis and therefore could usefully have been swapped for the opening three chapters on the genetics of nitrogen fixation in free-living bacteria found in volume 7 (also a little late to be useful since most of the data has appeared elsewhere by now). We find that *Rhizobium* genetics is in a primitive state by comparison.

The rest of volume 7 describes nitrogen fixation in symbiotic systems, both legumes and non-legumes. It includes sections on legume inoculation (reviews and research reports), legume field experiments (mostly research reports on plant yields versus inoculation or fertilisation), and reactions of legumes to environmental stress (including legume physiology). The final section includes non-leguminous root symbioses and the

aforementioned blue-green algal-plant associations. The negative results of chapter 39 should kill the oft-sought, oft-cited nitrogen fixing role of nodules found on certain tropical leaves.

On the whole these volumes contain an unprecedented collection of information and references. Access could have been improved by better indexing, especially since some chapter titles are misleading. Since the books will probably be bought as the set, cross-indexing would have been more useful than the multi-lingual tables of contents. They are burdened with the usual disadvantages of large-scale publishing enterprises in being too expensive and too late. The meeting was in 1973 and as a consequence neither volume refers to areas of more recent importance: the role of glutamine synthetase as a regulator of nitrogen fixation, fixation by *Rhizobium ex planta* (or even in callus culture) or nitrogen fixation by *Spirillum lipoferum* in maize plants.

Finally, volume 7 closes with excerpts from the meeting's last session which was a colourful, sometimes heated, sometimes amusing discussion of possibilities for extending nitrogen fixation to other plants. Unfortunately that atmosphere doesn't come through. The written version of Ray Valentine's comments that "exploratory work . . . is not necessarily expensive" just doesn't make it since what he really said was "doing this stuff will only cost a few bucks." □

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## New power horizons

G. R. Bainbridge

*Energy: The Solar Hydrogen Alternative.* By J. O'M Bockris. Pp. xviii+365. (Architectural Press: London, February 1976.) £13.50.

PRACTICAL scientists and engineers, energy planners and policy makers, to whom the author says his book is directed, will have no difficulty while reading it in coming to the conclusion that economic, large scale solar energy and hydrogen fuel systems are still many decades away. The uncertainties

about continuing supplies of fossil fuels, including the problems of extracting, transporting and applying them economically will lead to increasing research and development work into several alternatives: in particular, solar and hydrogen.

The students of energy conversion, the environmentalists and people concerned in an effort to prepare for the coming era of difficulty, in which a change in energy source has possibly to be made from the diminishing fossil



fuels, might reasonably be excused if they remain confused after trying to read the multi-faceted presentation from cover to cover. The book contains a bit of everything in energy; atomic, wind, geothermal, hydro and tidal illustrations are all martialled sequentially, as well as the main subjects of the book, to help to answer a whole host of questions about the rate at which abundant 'clean' energy might substitute for diminishing resources of coal, oil and natural gas. The oil and gas, warns the author, may be scarce and expensive within a few decades and the demise of coal may be only a few decades later. Nuclear fission and fusion systems will have their problems and may not be sufficient if the time before there is a real need for them is shorter than some people expect.

The propositions concerning doom in the 21st century due to increasing CO<sub>2</sub> and aerosols in the atmosphere, from the burning of fossil fuels, affecting world climate are brought out, as are the prospects of NO, CO and SO<sub>2</sub> causing substantial health hazards. The idea of the delayed and therefore necessarily nimble changes in technology and engineering being achieved to avoid catastrophe by providing

abundant new forms of energy, are not expected to be realised. Practical population control and the highly efficient recycling of materials to avoid shortages are not viewed hopefully. Experience indicates that modifications in the world energy mix and social behaviour patterns are slow and will continue to be so.

How soon in the future will ideas of the solar-hydrogen economy be strongly tested? Will the money, the manpower and materials necessary be made available by Governments for building large remote land-based, or offshore, atomic and solar power stations? If they can be, the scale of expenditure is bound to be limited. The reader may have doubts about this happening significantly in view of the recent slowing down of fission and fusion power research and development work. The stages outlined by Bockris seem then to be far too ambitious and to deviate too far from a main line of attack on the main solar electric and hydrogen production problems.

On these matters the reader is best left to form a personal judgement, to assist which some of the multitude of references so excellently given could be consulted, at a rate averaging about 1.6 per page of text. □

encyclopaedic survey by Christos Flytzanis of the Theory of Non-linear Susceptibilities. The author presents their phenomenological properties and evaluations in massive mathematical detail. Generalisations then give way to minute discussion of individual models relating to rarefied and condensed media. Some 500 references complete the review. The Measurement of Non-linear Optical Susceptibilities by Stewart K. Kurtz complements the previous theory. After a prefatory review of Gaussian beam optics and light propagation in anisotropic media, there follow practical descriptions of the absolute methods of phase matching, parametric fluorescence and Raman scattering; and the relative methods of Maker fringes, and wedges.

Introducing Part II (Non-linear Optical Processes). Two-Photon Absorption Spectroscopy by H. Mahr is a lucid account of the underlying physics of symmetry, polarisation and resonance applied to molecular and electronic transitions. Experimental results in solids, liquids and gases are surveyed. I. L. Fabelinskii reviews experiments and practical theory relating to the Stimulated Mandelstam-Brillouin Process. Studies of electrostrictive coupling, stimulated temperature process, non-linear absorption are related to a variety of stationary and transient phenomena. A short essay by C. L. Tang on Spontaneous and Stimulated Parametric Processes explains the basic theory and experimentation of parametric fluorescence, and discusses its applications. Concluding Part II, Chen-Shou Wang writing on The Stimulated Raman Process presents theoretical studies of the Raman coupling of pump and scattered light with mechanical mode excitation, both in forward and in backward transient stimulated scattering. Related effects and experimentations are briefly discussed.

Part III (Applications) opens with a substantial review by S. A. Akhmanov, A. I. Kovrygin and A. P. Sukhorukov of Optical Harmonic Generation and Optical Frequency Multipliers. This chapter concentrates on the theory and design of optical frequency multipliers, and divides into steady-state and transient generation, the latter including ultrashort laser pulses. In Optical Parametric Oscillators (Robert L. Byer), theory and experimentation are elegantly blended into a complete exposition of operation characteristics, devices and materials. In the final chapter by John Warner, the technique of Difference Frequency Generation and Up-conversion are briefly explained, the latter mainly for signal and image detection.

These volumes should form an authoritative text for some years.

P. G. Harper

## Solar connection

*Possible Relationships Between Solar Activity and Meteorological Phenomena.* Edited by W. R. Bandeen and S. P. Maran. Pp. ix+263. (NASA: Washington, DC, 1975; sold by US Government Printing Office.) \$4.00.

THIS book marks the official publication of the proceedings of the NASA Symposium on possible relationships between solar activity and meteorology held at the Goddard Space Flight Center in November 1973. I share the disappointment of NASA's Scientific and Technical Information Office concerning the delay in publication, as this was clearly an important conference in defining the starting ground for much of the current work on solar-weather links. Publication in 1974 would have been of great value to those investigating these links who were unable to attend the conference. Two years later, its value is much diminished.

Since the proceedings have been available in preprint form since June 1974, at no charge, and the book costs \$4, it might seem that the only recommendation for this edition is that it is more compact than the preprint. The papers are often brief to the point of superficiality and in many cases very subjective, whereas the field of study is very rapidly becoming a much tighter and more

objective discipline than it was up until recently. S. I. Rasool aptly summarises the conference: "What we do not want is a symposium where there are 5-minute presentations and where one cannot ask a question without a microphone". Nor, in 1976, do we want a book which reports uncritically the proceedings of such a symposium on this important topic.

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## Authoritative text on quantum electronics

*Quantum Electronics: A Treatise.* Edited by Herbert Rabin and C. L. Tang. Vol. 1: Non-Linear Optics, Part A. Pp. xi+472+9. \$35; £17.50. Vol. 1: Non-linear Optics Part B. Pp. ix+473-753. \$22.50; £11.25. Academic: New York and London, December 1975.)

THE nine chapters of these long-awaited volumes cover substantial ground, enough, as Bloembergen remarks in a general introduction, to catch the spirit of non-linear optics without exhausting its content. Common chapter organisation and notation frequent cross-referencing, a universal index, all in attractive format, make for good consultation.

Part I (Non-linear Optical Susceptibilities) is mostly taken up with an

## Mathematical explanation

*Introduction to Mathematical Biology.* By S. I. Rubinow. Pp. xiii + 386. (Wiley-Interscience: New York and London, December 1975.) \$27.00; £13.55.

THIS book is based on a course designed to develop in graduate biology students at Cornell University an awareness of and familiarity with some mathematical techniques and methods applied to biology. Apart from brief mention of the application of the least squares method to linear regression and the extraction of the parameters of multi-exponential processes by the 'peeling' method, statistical and numerical analytical techniques for processing experimental data are not featured. The author clearly believes, however, that mathematical biology should be firmly based on experimental observation. Consequently, no attempt is made to describe methods involving analogue or digital computers for handling speculative mathematical models of biological systems.

The book deals with the mathematical explanation of five selected biological phenomena in some depth: cell growth, enzyme kinetics, tracers in physiological systems, biological fluid dynamics and diffusion in biology. The style is economical so that, for example, steady-state and pre-steady-state kinetics, cooperativity and allostery and the effects of inhibitors and modifiers are treated in 53 pages in the chapter on enzyme kinetics.

Where possible, the author applies fundamental concepts to a variety of biological problems. For example, an explanation of Fick's laws of diffusion leads to consideration of topics as diverse as the relationship between diffusion constant and molecular weight, pheromone interaction between animals, membrane transport, diffusion through a slab, axonic ion flow, ultracentrifugation and diffusion across capillary walls. Three other features of the book deserve mention. When a new term is introduced and defined, it is printed in bold-faced type which will facilitate revision by students. Each chapter ends with a number of problems of graded mathematical difficulty. The solutions with full explanations are given towards the end of the book.

Finally, although the author states that this is not a textbook in mathematics, there are two useful mathematical appendices for readers who have forgotten basic techniques such as the handling of matrices and determinants. Unfortunately, stu-

## PMO theory

*The PMO Theory of Organic Chemistry.* By Michael J. S. Dewar and Ralph C. Dougherty. Pp. xiii + 576. (Plenum: New York, 1975.) \$14.50.

IN the preface, the authors disclaim any intention of rewriting the whole of organic chemistry in terms of perturbational molecular orbital (PMO) theory. The text illustrates principles of such widespread application that it seems that only time has prevented them from so doing. The first three chapters introduce the theory of the method in a most readable manner. The principles are well presented, and the material could stand up in its own right as a separate text. The bulk of the text comprises three chapters with far-reaching titles—Chemical Equilibria (ch.4), Chemi-

cal Reactivity (ch.5) and Light Absorption and Photochemistry (ch.6). The final chapter is concerned with transient ions, and seems too specialised for the general audience at whom the work as a whole is aimed. Inevitably some of the material will provoke controversy, and there is a rather cavalier treatment of alternative approaches to some of the problems. For example, the summary dismissal of the Hoffmann-Woodward approach to Pericyclic Reactions, and the absence of any reference to R. W. Taft in the section dealing with substituent effects would be remarkable if it were not so predictable. The book succeeds admirably in its stated aim of demonstrating the power of the PMO method, and does so in such a readable manner that it will be appreciated by a wide audience.

L. Phillips

dents of the biological sciences who could and should read this book are likely to find the price a deterrent. It deserves a place in science libraries and hopefully will be well thumbed with use by students and lecturers.

D. T. Elmore

## Infrared astronomy

*Infrared: The New Astronomy.* By David A. Allen. Pp. 228. (Keith Reid: Sheldon, Devon, November 1975.) £6.50.

THIS is an unusual book, a semi-popular one written completely from the viewpoint of the observational astronomer. It charts the rise of a new field, infrared astronomy, with which the author has been actively involved for a decade. After introductory chapters outlining a few background ideas, the techniques of observation (formidable if the only dewar you have ever handled is a thermos flask), the main observing sites, and the early history, the book discusses the different types of source seen in the infrared: solar system objects, stars and protostars, and galaxies and quasars. All are discussed on the basis of whether they are detectable in the infrared, and more particularly whether they are unusually strong emitters there. This seems to be the weakness of the observer's viewpoint, especially when he is specialising in a fairly narrow range of wavelengths (most of the book concerns the range 1–20  $\mu\text{m}$ ). No theoretical framework is provided which explains why different types of star or galaxy are of interest. So this is a book which requires a rather extensive knowledge

of astronomy to appreciate its qualities.

The chapters on the different types of star which radiate in the infrared, either because they are cool or more often because they are partially shrouded in dust, are outstanding, and constitute a valuable review of, and introduction to, the field.

That on galaxies and quasars, on the other hand, opens weakly: "A galaxy is some breccia constructed of all the types of object we have encountered in the last four chapters". No real effort is made to distinguish between different types of galaxy. Under "Compact Galaxies" we find I Zw 172+50, which is also a BL Lac-type object: the "Markarian galaxies" mentioned are also, in fact, Seyferts. And the chapter ends with a decidedly casual (and even for June 1974, inaccurate) account of the microwave background.

If Dr Allen were writing the book today, I think he might devote a larger fraction to wavelengths between 30  $\mu\text{m}$  and 1 mm, where many of his sources emit most of their energy. He might also devote more space to the clouds of dust and molecules out of which form the young stars on which he is so expert.

This is a book for the professional astronomer (for whom an excellent feature will be the comprehensive list of references) or the knowledgeable amateur. How the style appeals to you will depend on your taste for extravagant metaphor. For example, synchrotron radiation is explained as "those piercing shrieks emitted by high energy electrons when they embark on their helter-skelter paths in magnetic fields".

M. Rowan-Robinson



## Lemurs

*Lemur Biology*. Edited by Ian Tattersall and Robert W. Sussman. Pp. xiii + 365. (Plenum: New York and London, 1975.) \$27.54.

THIS is a progress report of recent studies. To emphasise the breadth of modern interest in lemurs, studies from several disciplines have been drawn together. There are five sections: an introduction to lemur biology (the history of the study of lemurs; the Madagascan environment); systematics and evolution (comparative studies of chromosomes and teeth); morphology and physiology (studies of cranial anatomy, postcranial skeleton and body temperature); behaviour and ecology (studies of several little-known lemurs including *Indri*, *Phaner*, *Haplemur* and *Propithecus*); and an epilogue on conservation and extinction. The editors emphasise that "the interpretations in each of these fields are dependent on knowledge of each of the others". It therefore seems reasonable to expect them to promote

the cross-fertilisation of disciplines by ensuring that individual contributions are understandable to the non-specialist. Although most papers are admirable in substance they leave the non-specialist confused by repeatedly using specialised jargon (for example, terms from cytogenetics, dental and postcranial morphology, behaviour) which is either inadequately defined or not defined at all. Most readers, being less familiar than the authors with Madagascar, may also find difficulty in following the text because of the lack of maps showing topographical reference points and the distribution of species. The usefulness of this style of book is limited unless it is something more than a collection of papers of the type found in specialist journals.

In spite of those criticisms I enjoyed *Lemur Biology*. Its breadth of coverage is refreshing and instructive. There has clearly been a delay in publication and the preface sensibly records the most recent developments.

John M. Deag

## Down to Earth

*Man's Relation to the Universe*. By Bernard Lovell. Pp. vi+118. (Freeman: San Francisco and Reading, November 1975.) \$5.95.

DURING 1973, Professor Bernard Lovell delivered a series of lectures on astronomy and space research to students at Buffalo University. The content of these lectures is available in an informative little book which carries the somewhat misleading title of *Man's Relation to the Universe*.

Indeed the first chapter is very much down to Earth, with an unusual and entirely welcome discussion about the financial cost of space-age astronomy. We are told such horrors as the (certainly astronomical) cost—\$400 million—of the Soviet 236-inch telescope, although to put it all in perspective, the UK government turns out to spend only 0.02% of the gross national product on all forms of astronomical research.

Stepping into near space Professor Lovell skillfully summarises the results gleaned by the recent space probes to Mercury, Venus, Mars and Jupiter. The explosion of information about these planets is bewildering, but the author delivers a well-balanced and cautious appraisal of contentious issues, such as the 'water on Mars' controversy, but without losing a sense of excitement and enthusiasm.

Moving farther afield, stellar and

galactic evolution are described, and modern discoveries such as quasars, pulsars and (possibly) black holes all get a mention. The discussion is somewhat formal, but never dull or over extended. In spite of the title, there is very little hard philosophy, except on cosmological issues such as the big-bang creation event.

I very much enjoyed reading this book. Laymen of a scientific inclination may find rather too much technical discussion to cope with the content without preparation. Scientists and students will find it a straightforward and engaging introduction to the techniques and results of modern astronomical research.

Paul Davies

## Aquatic fungi

*Recent Advances in Aquatic Mycology*. Edited by E. B. Gareth Jones. Pp. 749. (Elek Science: London, December 1975.) £21.

THE idea for this volume grew from the success of seven sessions devoted to aquatic fungi at the First International Mycological Congress held in Exeter in 1971, and some of the chapters are papers presented at the Congress. It is disappointing that it has taken four years for the book to appear, so that some of the advances are by now hardly recent.

About half of the book, Section I, is devoted to marine fungi; the rest, Section II, to freshwater forms. The marine fungi are considered under two arbitrary divisions—A, Ascomycetes, Fungi Imperfecti and Basidiomycetes; and B, Lower Fungi. Section IA has chapters on lignicolous fungi, mangrove fungi, oceanic yeasts, fungal degradation of oil, physiology, and cytochemistry of spores. I particularly enjoyed the account by J. W. Fell of oceanic yeasts, some of basidiomycetous affinity, which brought together, in a coherent way, material from widely scattered literature of a group which will be unfamiliar to many. Section IB, mostly covering zoosporic forms, includes a general account of marine 'phycomycetes', an essay by F. K. Sparrow on the present status of classification in biflagellate fungi (not exclusively marine), and chapters on fungal diseases of marine animals, physiology of marine 'phycomycetes', fine structure, and ecology. The freshwater Section is also divided into two arbitrary divisions. The section on Ascomycetes, Fungi Imperfecti and Basidiomycetes has chapters on morphology and biology, fungi from cooling towers, sewage, an interesting account of the role of Hyphomycetes as intermediaries of energy flow in streams, and on the interactions between river fungi and DDT. The 'lower fungus' section includes chapters on the morphology of Chytridiomycetes in relation to substrates, fungi on algae, ecology, physiology, aspects of fish disease, ultrastructure, and a valuable review of the Trichomycetes by R. W. Lichtwardt. Aquatic Actinomycetes, although accepted as prokaryotic, are included in a useful chapter by L. G. Willoughby.

The general impression of the book, apart from the uneven quality inevitable in symposium-type publications, is that it will provide useful reference material for those working on aquatic fungi. The photographic illustrations leave much to be desired, especially with respect to size, layout and quality of reproduction. The use of unglazed paper and the faint printing has rendered many of the electron micrographs virtually useless, as is shown in the otherwise excellent article by Heath on ultrastructure of freshwater Phycomycetes. Many of the plates have obviously been made up by their authors to fill a whole page, but have been reduced by the publishers to take up only a small fraction. Much of the space 'saved' by this unfortunate economy has been wasted by the use of large print and author citations in voluminous tables. Moreover, there are numerous misprints. The book will prove far too expensive for many individuals to buy.

John Webster

# obituary

The news of **Michael Polanyi's** demise caused deep sadness in the hearts of his many friends and admirers. There were, and are, few people in our century whose interests were as extended as were his, whose friends were as devoted to them as were those of "Misi".

Polanyi was born in Budapest, Hungary, on March 11, 1891. He took his degree as an MD in Budapest at the age of 22, but wrote his first paper (on the hydrocephalic liquid) long before that, at the age of 19. His interest then centred on biology, principally human biology, but his articles soon became sprinkled with references to, and conclusions drawn from, physical chemistry, and, in particular, the second law of thermodynamics. The first such paper was written in collaboration with a life-long friend, J. Baron, but the other papers written during the first world war, when he served in the army, were by him alone. They were concerned not only with applications of the second law, but also with the foundations of the third law, then called Nernst's Theorem. His last article during the war period, however, was on a subject which remained long close to his interest: the theory of adsorption.

Soon after the end of the war, Polanyi moved to Germany—for a year to Karlsruhe, where he met his wife, then to Berlin where he worked first at the Kaiser Wilhelm Institut für Faserstoffchemie (chemistry of fibres), and then at Haber's Institute, the institute for physical and electrochemistry. He moved to Manchester, England, in 1933, when Hitler came to power, a reason very similar to that which had originally prompted him to leave Hungary.

The breadth of Polanyi's interests manifested itself in his days as a scientist by the wealth of subjects to which he made important contributions. Several of them continued to attract his interest virtually all through his scientific career. His first articles on the subject mentioned before, the adsorption of gases to solid surfaces, were written in 1914 and 1916, the last one referring to this subject in 1946. His chief interest was, however, concentrated on the rate of chemical reactions. He contributed decisively both to the theory and to the development of experimental methods to investigate them. His first paper on the



subject was written in 1920, a theoretical paper which was later fully justified by quantum mechanical considerations in 1925 and his concluding paper on this topic dates from 1949. In addition to these, he contributed significantly to our understanding of the strength of materials and the role of the crystalline glide-planes in this connection, to the X-ray analysis of fibres and crystals, also of poly-crystalline materials and, finally, to simple chemistry (and perhaps to other subjects which this writer fails to recall).

Two thirds of Polanyi's scientific articles written after 1920 (he published 201 such articles) were published jointly with collaborators, some of whom have acquired world fame. He not only stimulated interest in his collaborators, he also established close personal relations with most of them and they maintained their devotion to him and his ideas throughout his life. This applies also to the present writer.

**Eugene P. Wigner**

Were it not that he was one of the great polymaths of this century, it would be surprising, as it was apparently regrettable to some of his science colleagues, that Michael Polanyi, after coming to the University of Manchester in 1933 as Professor of Physical Chemistry with a great international reputation in science, should in 1948 have been appointed as Pro-

fessor of Social Studies. Yet it is intriguing to speculate as to why he made the decision, with the general approval of his University and the warm welcome of the economists there, to shift the balance of his intellectual interests. Running through his writings in economics—USSR Economics in 1935; Patent Reform 1944; Full Employment and Free Trade in 1945; Profits and Polycentricity in 1946; and The Span of Central Direction 1948—there seems to be one leading strand: how best to reconcile the safeguarding of individual liberty with the controls upon the individual inseparable from a complex and organised society or, as he succinctly put it, the relation between spontaneous and social order.

Within two years of reaching Manchester he had produced his small but pathbreaking book *USSR Economics*. It was the result of a flash of understanding of the kind which genius enjoys. He saw that the idea of rational planning of the whole of the economic system by Governments was a complete illusion. Then, with remorseless logic, he asked himself: if central economic planning is indeed a chimera, what is it that Governments which set out to engage in it, are in fact doing? And, prophet as he was, he foresaw the answers. In countries, such as Russia, where the ideas were then being ruthlessly pursued, the result would be the creation of a slave state. In other Western countries (and he had in mind among these Britain) where individual freedom was more deeply entrenched and tenaciously defended, the attempts of Governments at central planning would simply lead to great muddle, with increased public intervention in industrial investment, economic debility, widespread price controls and inflation.

This central discovery he filled out in his later writings. He sought to explain by word, and by an ingenious film, the subtleties and efficacy of free market operations. Admitting to weaknesses in a system of private enterprise he developed ideas, along Keynesian lines, for the prevention of mass unemployment and for the more active encouragement of technical advance. He displayed in novel ways how, as an industrial organisation grows in size, it tends towards inefficiency in operation. He argued for the crucial role of profits in a free economy: "There exists no fundamental alternative to the system



of money-making and profit-seeking".

After 1948 his mind moved into more abstract and philosophical fields. But in a remarkably short time he had left behind him a set of writings which economists in the future will turn to and find rich and stimulating.

**John Jewkes**

Though Michael Polanyi wrote a number of studies in the philosophy of science, his contribution rests mainly upon a single work, *Personal Knowledge*, first published in 1958. In both style and content it ran strongly counter to the logicist trends of the time, and was not easily assimilated into professional philosophy of science, though it was greatly appreciated by many working scientists. Written in a limpid, urbane style, drawing upon a

wider range of experience than was usual in philosophy of science, it might be properly thought of as setting a trend towards 'science criticism', bearing a somewhat similar relation to science that literary criticism does to literature.

Two philosophical themes dominated this work, and all Polanyi's reflection on the nature of science; the theme of 'tacit knowledge' and the theme of the analogy of perception. Polanyi never tired of emphasising in many contexts that we know 'more than we can tell', a truth not only of the practical arts but as he persuaded many of his readers, of the intellectual arts as well. Explicit scientific knowledge rests for its intelligibility and grounding upon an undetermined basis of tacit knowledge, which if made explicit rests again

upon its own basis of the tacit. Explicit knowledge is public knowledge, but tacit knowledge is private, and both sorts of knowledge are encompassed within science.

The relation of explicit knowledge to its penumbra was to Polanyi either identical with or perhaps only similar to the relation between focal and subsidiary awareness in perception, attending from something to something else. He elaborated this metaphor in a number of works.

That science was a part of society, that it was within the larger society a society itself, but a society with a peculiarly morally demanding mode of being, was both exemplified by the man himself, and the central message of his philosophical work.

**Rom Harré**

## announcements

### Appointments

**Dr Peter W. Likins**, of the University of California, Los Angeles has been named dean of Columbia University's School of Engineering and Applied Science.

**Dr Michael A. Moore** of Sussex University to Professor of Theoretical Physics at the University of Manchester.

**Dr R. J. Ellis** to a personal Professorship of Biological Sciences at Warwick University.

**Dr Hugh Burkhardt** to Professor of Mathematical Education at Nottingham University.

**Sir John Habakkuk**, Vice-Chancellor of the University of Oxford, has been elected Chairman of the Committee of Vice-Chancellors and Principals of the Universities of the United Kingdom for the year 1976-77.

### Awards

**Professor N. Kurti** of the University of Oxford has been made a Chevalier de la Légion d'Honneur.

**Professor C. T. Dollery** of the Royal Postgraduate Medical School has been made a Chevalier de l'Ordre du Mérite.

**Dr Charles Elton** of the University of Oxford, **Dr Rene Dubos** of the Rockefeller University and **Dr Abel Wolman** are the recipients of this year's Tyler Ecology Award.

### International meetings

May 27-28, **The Handling of Toxicological Information**, Bethesda (Dr Arthur A. Wykes, Toxicology In-

### Person to Person

Information is required on national and international efforts to popularise science, on famous science writers from all over the world and on awards given to science writers. Scientists who communicate to laymen please send their opinions to Zaka Imam, Assistant Editor, Science Reporter, Council of Scientific and Industrial Research, Rafi Marg, New Delhi 110001, India.

Astronomer attending international meeting wishes to exchange 3-bedroomed house (sleeps 6 and baby) in Sussex for house or flat (sleeping 5 and baby) near Grenoble, France, for one month during August 1976 (until about 3rd September—meeting ends on 2nd). Enquiries to Dr R. C. Smith, 38 The Avenue, Lewes, Sussex BN7 1QU, England.

There will be no charge for this service. Send items (not more than 60 words) to Martin Goldman at the London office. The section will include exchanges of accommodation, personal announcements and scientific queries. We reserve the right to decline material submitted. No commercial transactions.

formation Program, National Library of Medicine, 8600 Rockville Pike, Bethesda, Maryland 20014).

June 2-4, **Frequency Control**, Atlantic City, N.J. (Dr John R. Vig, USAECOM, ATTN:DRSEL-TL-MF, Ft. Monmouth, N.J. 07703).

July 17-22, 1977, **Macromolecules**, Dublin (Symposium Officer, Macro

Dublin 77, Institute for Industrial Research and Standards, Ballymun Road, Dublin 9, Ireland).

September 6-9, **Structure and Kinetic Approach of Plasma Membrane Functions**, Grignon, France (Deadline for application: May 15) (Dr Alain Paraf, Station de Virologie et d'Immunologie, Route de Thiverval, 78850 Grignon-Thiverval, France).

September 22-23, **Laboratory Animal Housing**, Washington DC (E. W. Grogan, Executive Secretary of ILAR, National Research Council, 2101 Constitution Avenue, N.W., Washington, DC 20418).

September 22-24, **Congress of the European Chemoreception Research Organisation**, Reading, UK (Deadline for registration: June 30) (Dr G. H. Dodd, Department of Molecular Sciences, University of Warwick, Coventry CV4 7AL, UK).

October 11-15, **Structure and Uses of Biopolymers** (Professor Alan G. Walton, Department of Macromolecular Science, Case Western Reserve University, Cleveland, Ohio 44106).

October 18-20, **Platinum Co-ordination Complexes in Cancer Chemotherapy**, Dallas (Deadline for abstracts: June 30) (Third International Symposium on Platinum Co-ordination Complexes in Cancer Chemotherapy, Wadley Institutes of Molecular Medicine, 9000 Harry Hines, Dallas, Texas 75235).

November 9-10, **Techniques for the Retrieval of Chemical Information**, London (Dr John F. Gibson, The Chemical Society, Burlington House, London W1V 0BN, UK).